SLED DOGS AS A SENTINEL AND MODEL FOR NUTRITIONAL AND PHYSIOLOGICAL ADAPTATION IN THE CIRCUMPOLAR NORTH

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By

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Abstract

Sled dogs were investigated as a sentinel in studying adaptation to the circumpolar north. Exploratory data were collected to study the characteristics of melatonin and thyroid hormone and revealed an impact of day length, exercise and thermoregulation on production. Before western diets, circumpolar people had a low incidence of obesity, diabetes, and cardiovascular disease. Contrary to the risks associated with a high fat, high protein diet, health benefits can be attributed to a diet rich in omega-3 fatty acids and antioxidants, offered from subsistence foods. While subsistence diets have been shown to provide substantial health benefits, there are also risks associated with them as a result of industrialization and the widespread distribution of chemicals in the environment. Native people and their sled dogs are exposed to a variety of contaminants that have accumulated in the fish and game that they consume. The sled dogs in these villages are maintained on indigenous food, primarily salmon, and therefore they can be used as models for researching the effects that a subsistence diet might have on immune parameters. Several biomarkers of immune function and inflammation were measured in village sled dogs along the Yukon River. A reference kennel, maintained on a nutritionally balanced commercial diet, was also measured in all projects for comparison. The health indicators such as antioxidant status were inversely correlated with mercury exposure.

Dedication

This thesis is dedicated to the two people who have supported, encouraged, and championed me throughout my life; my best friends, my confidants, my backbone, my mentors, my heroes— my parents. This is also in memory of my loyal and unquestioning companion, who followed me in all my endeavors and almost made it through this one— my dog, Curtail.

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List of Abbreviations

AA	Arachidonic acid
CON	Control dogs
COX	cyclooxygenase
CRP	C-reactive protein
cTSH	Thyroid stimulating hormone
CVAF	Cold vapor atomic fluorescence spectrometry
DHA	docosahexaenoic acid
EPA	Eicosapentaenoic acid
EX	Exercise dogs
FT3	Free T_3
FT4	Free T_4
HETEs	Hydroperoxy-eicosatetraenoic and hydroxyeicosatetraenoic acid
IL-1	Interleukin-1
IL-6	Interleukin-6
LOX	lipoxygenase
LT	Leukotrienes
LTB_4	Leukotriene B_4
PG	Prostaglandins
SAD	Seasonal affective disorder
SCN	Suprachiasmatic nuclei

T_3	Triiodothyronine
T_4	Thyroxine
TAP	Total antioxidant power
TBG	Thyroid hormone binding globulin
THg	Total mercury
TNF-α	Tumor nucrosis factor-α
TT3	Total T_3
TT4	Total T_4
TX	Thromboxanes
SAD	Seasonal affective disorder
SCN	Suprachiasmatic nuclei

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Chapter 1

Introduction

1.1 Sled dogs as a model

Dogs have become a popular model for immune function, nutrition, exercise, toxicology and cognitive disorders (Strasser et al., 1993; Adams et al., 2000; Greeley et al., 2001; Milgram et al., 2002; Balagangatharathilagar et al., 2006) because they have key features associated with cognitive dysfunctions, beta-amyloid pathology, and oxidative damage similar to that of humans (Milgram et al., 2002). Sled dog mushing, once used primarily as a means of transportation, has evolved into a popular national and international sport. This lends to diversity in climate, diet and location, providing great research opportunities. Sled dogs are unique research models because they can be found in a large, genetically homogenous sample size. Because of this, the effects of diet, exercise, disease, and environment can be observed on the immune, cardiovascular and endocrine systems.

Sled dogs in northern climates are often exposed to the same environmental hazards as their human counterparts (Hansen and Danscher, 1995). In many Alaskan villages sled dogs are still a fundamental part of a traditional lifestyle, used for trapping, packing and transportation. Most of these villages are small settlements, established on or near rivers to facilitate travel and to gather food. The diet of both the natives and their sled dogs in Alaska is often comprised of a variety of wild game, fish and marine mammals (Andersen, 1992; McGrath-Hanna et al., 2003). Before the arrival of western diets, circumpolar people had a low incidence of obesity, diabetes, and cardiovascular disease. Researchers attribute this to the loss of a typical subsistence diet, abundant in polyunsaturated fatty acids and antioxidants (Adler et al., 1994; Mozaffarian and Rimm, 2006).

For Alaskan Natives that still live a subsistence lifestyle, contaminant exposure may be a health risk. Mercury is transported to the circumpolar north by means of wind, ocean currents and migrating animals. Concentrations of mercury increase up trophic levels from prey to predator, in a process called biomagnification (Dehn et al., 2006; Tyrell, 2006). Native people are more exposed to contaminants because of their reliance on marine mammals (Tyrell, 2006) and fish. Sled dogs provide a large homogenous sample size for studying the impact of subsistence lifestyles and subarctic environments on immune and endocrine functions.

1.2 Mercury exposure

Contaminants exposure has been linked to neurological illness, cancers, depression, cardiovascular disease, impaired immune function and endocrine disruption (Porterfield, 2000; Bélanger et al., 2006; Tyrell, 2006). Several biomarkers associated with these diseases were measured in the following studies. Immune parameters and total mercury concentrations were measured in subsistence fed sled dogs in several villages along the Yukon River. Comparisons were made between these villages and a reference kennel, located in Salcha (65°N), Alaska. An additional kennel was sampled in North Creek, NY (45°N).

1.3 Cross-latitudinal, diurnal and seasonal production in melatonin and thyroid hormone production

Melatonin and thyroid hormone production, both involved with immune and endocrine function, were also measured in sled dogs at different latitudes. Light cues detected through photoreceptor cells in the retina inhibit melatonin secretion via the suprachiasmatic nuclei (SCN) and therefore melatonin production is proportional to night length (Lincoln, 2006; Rajaratnam et al., 2006), giving melatonin both a diurnal and a seasonal rhythm which can vary between populations at differing latitudes (van Oort et al., 2006; Levine et al., 1994). The northern location of Fairbanks, Alaska can experience anywhere from 21 hours of daylight in the summer to 4 hours in the winter. This dramatic change is expected to affect the body's internal clock by dictating the amount of melatonin produced.

The timing and amplitude of melatonin production has been implicated in seasonal affective disorder (SAD) (Danilenko et al., 1994; Nelson et al., 2002). Light therapy has been shown to alleviate symptoms of depression in the winter (Danilenko et al., 1994; Leppamaki et al., 2002). Exercise alone or in combination with light therapy has been reported to have a positive impact on seasonal mood slumps (Leppamaki et al., 2002). Melatonin production was monitored in sled dogs as a consequence of season, daylight exposure, sampling time and exercise.

Thyroid hormone production is regulated by the hypothalamic-pituitary-thyroid hormone negative-feedback axis (Nelson et al., 2002). The main form of thyroid hormone produced by the thyroid gland is thyroxine (T_4). Extrathyroid tissues regulate the deiodization of the "prohormone" T_4 to the active form of the hormone, triiodothyronine (T_3). Small increases in T_4 and T_3 suppress the production and secretion of thyroid stimulating hormone (cTSH). Thyroid hormones, in serum, are either in a free form or bound to carrier proteins. Hypothyroidism is the most common endocrine disorder in dogs and very difficult to diagnose (Ferguson, 1994; Kaptein et al., 1994).

Documented deviations in human thyroid hormones have resulted from seasonal changes (Levine et al., 1995; Plasqui et al., 2003), exercise (Panciera et al., 2003), diurnal variations (Kaptein et al., 1994; Surks et al., 2005) and possibly as a consequence of light (Levine et al., 1994). Cold environments are known to heighten thyroid activity (Plasqui et al., 2003), but the mechanism is uncertain. Fluctuations in thyroid hormone are not limited to seasonal cycles but diurnal variations are also present. We observed effects of thermoregulation, sampling time, day length and season on thyroid hormone production in sled dogs. Alaskan sled dogs on the winter solstice are exposed to only 3.5 hours of low light but these same dogs in the summer are exposed to 21 hour of daylight. Results presented here agree with previous reports suggesting sled dogs need a lowered reference range for thyroid hormone concentrations. Additionally, standardizing sampling time would improve comparative studies and advance the field.

1.4 Summary

Chapter 2 reports seasonal and diurnal variations in melatonin production in exercising and non-exercising sled dogs. Chapter 3 reports cross-latitudinal, seasonal and diurnal comparisons in thyroid hormone concentrations in sled dogs. Chapter 4 discusses the linkages between mercury exposure in Yukon Rivers sled dogs and human subsistence food systems. And lastly, Chapter 5 illustrates relationships between immune parameters and mercury exposures in subsistence fed sled dogs along the Yukon River. Using sled dogs as a model should enhance our understanding of how exercise and diet can impact immune and endocrine function in climates with extreme temperatures and light variations.

1.5 References

Adams B, Chan A, Callahan H, Siwak C, Tapp D, Ikeda-Douglas C, Atkinson P, Head E, Cotman C, Milgram N. Use of a delayed non-matching to position task to model age-dependent cognitive decline in the dog. *Behav Brain Res* 2000; 108: 47-56.

Adler AI, Boyko EJ, Schraer CD Murphy NJ. Lower prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption among Alaska Natives. *Diabetes Care* 1994; 17: 1498-1501.

Andersen DB. The use of dog teams and the use of subsistence-caught fish for feeding sled dogs in the Yukon River drainage, Alaska. Alaska Department of Fish and Game Technical Paper No. 210; 1992.

Balagangatharathilagar M, Swarup D, Patra RC, Dwivedi SK. Blood lead level in dogs from urban and rural areas of India and its relation to animal and environmental variables. *Sci Total Environ* 2006; 359: 130-4.

Bélanger MC, Dewailly E, Berthiaume L, Noël M, Bergeron J, Mirault ME, Julien P. Dietary contaminants and oxidative stress in Inuit of Nunavik. *Metabolism* 2006; 55: 989-95.

Danilenko KV, Putilov AA, Russkikh GS, Duffy LK, Ebbesson SO. Diurnal and seasonal variations of melatonin and serotonin in women with seasonal affective disorder. *Arct Med Res* 1994; 53: 137-45.

Dehn LA, Follmann EH, Thomas DL, Sheffield GG, Rosa C, Duffy LK, O'Hara TM. Trophic relationships in an Arctic food web and implications for trace metal transfer. *Sci Total Environ* 2006; 362(1-3): 103-23.

Ferguson DC. Update on diagnosis of canine hypothyroidism. *Vet Clin North Am Small Anim Prac* 1994; 24(3): 515-39.

Greeley EH, Ballam JM, Harrison JM, Kealy RD, Lawler DF, Segre M. The influence of age and gender on the immune system: a longitudinal study in Labrador Retriever dogs. *Vet Immunol Immunopathol* 2001; 82: 57-71.

Hansen J, Danscher G. Quantitative and Qualitative Distribution of Mercury in Organs from Arctic Sledgedogs: An Atomic Absorption Spectrophotometric and Histochemical Study of Tissue Samples from Natural Long-Termed High Dietary Organic Mercury-Exposed Dogs from Thule, Greenland. *Pharmacology and Toxicology* 1995; 77: 189-195.

Kaptein EM, Hays MT, Ferguson DC. Thyroid hormone metabolism: a comparative evaluation. *Vet Clin North Am Small Anim Prac* 1994; 24(3): 431-63.

Leppamaki SJ, Partonen TT, Hurme J, Haukka JK, Lonnqvist JK. Randomized trial of the efficacy of bright-light exposure and aerobic exercise on depressive symptoms and serum lipids. *J Clin Psychiatry* 2002; 63(4): 316-21.

Levine ME, Milliron AN, Duffy LK. Diurnal and seasonal rhythms of melatonin, cortisol and testosterone in interior Alaska. *Arct Med Res* 1994; 53: 137-145.

Lincoln GA. Decoding the nightly melatonin signal through circadian clockwork. *Mol Cell Endocrinol* 2006; 252: 69-73.

Milgram NW, Zicker S, Head E, Muggenburg B, Murphey H, Ikeda-Douglas C, Cotman C. Dietary enrichment counteracts age-associated cognitive dysfunction in canines. *Neurobiology of Aging* 2002; 23: 737-745.

McGrath-Hanna N, Greene D, Tavernier R, Bult-Ito A. Diet and mental health in the Arctic: Is diet an important risk factor for mental health in circumpolar peoples? - a review. *Int J Circumpolar Health* 2003; 62: 228-241.

Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006; 296(15): 1885-99.

Nelson RJ, Demas GE, Klein SL, Kriegsfeld LJ. Seasonal patterns of stress, immune function, and disease. (2002) Cambridge University Press, Cambridge, United Kingdom.

Panciera DL, Hinchcliff KW, Olson J et al. Plasma thyroid hormone concentrations in dogs competing in long-distance sled dog race. *J Vet Intern Med* 2003; 17(4): 593-6.

Plasqui G, Kester ADM, Westerterp KR. Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. *Am J Physiol Endocrinol Metab* 2003; 285: E338-343.

Porterfield SP. Thyroidal dysfunction and environmental chemicals—potential impact on brain development. *Environ Health Perspect* 2000; 108 Suppl 3: 433-8.

Rajaratnam SM, Dijk DJ, Middleton B, Stone BM, Arendt J. Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *J Clin Endocrinol Metab* 2006; 88(9): 4303-9.

Strasser A, Niedermuller H, Hofecker G, Laber G. The effect of aging on laboratory values in dogs. *Zentralbl Veterinarmed A* 1993; 40(9-10): 720-30.

Surks MI, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab* 2005; 90(9): 5489-96.

Tyrell M. Making sense of contaminants: a case study of Arviat, Nunavut. *Arctic* 2006; 59(4): 370-80.

Van Oort, B.E., Tyler, N.J., Gerkema, M.P., Folkow, L., and Stokkan, K.A. Where clocks are redundant: weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften* 2006; 94(3):183-94.

Chapter 2

Seasonal and diurnal melatonin production in exercising sled dogs*

2.1 Abstract

Melatonin is a hormone that is released from the pineal gland into the blood stream and is controlled by nerve impulses from the suprachiasmatic nuclei. Melatonin synthesis, which is inhibited by light on the mammalian retina, peaks in plasma concentrations during the night. Though still a subject of intense research, melatonin in mammals is known to effect the reproductive system, thyroid function, and adaptations to seasonal changes. Sled dogs in Fairbanks, Alaska (65° N) can be exposed to anywhere from 21 hours of daylight in the summer to 4 hours in the winter. While light may be the primary factor influencing melatonin production, we hypothesized that exercise may also affect melatonin production. In the current study, sled dogs were used to study seasonal and diurnal variation is melatonin production. Sled dogs by nature are elite athletes and therefore exercise was a focus in the study. Both exercise and non exercise dogs from 2 distinct latitudes were used. The peak in melatonin production was prolonged in high latitude dogs (65° N), compared with lower latitude dogs (45° N). Dogs at both latitudes show a reduction in peak melatonin levels with exercise, and winter melatonin levels in both locations were higher than the summer. Surprisingly, sled dogs in Alaska had lower

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melatonin levels than sled dogs in New York.

2.2 Introduction

The release of melatonin mostly at night by the pineal gland in the brain is controlled by nerve impulses from the suprachiasmatic nuclei (SCN). Light cues detected through photoreceptor cells in the retina inhibit melatonin secretion via the SCN and therefore melatonin production is proportional to night length (Lincoln, 2006; Rajaratnam et al., 2006), giving melatonin both a diurnal and a seasonal rhythm which can vary between populations at differing latitudes (van Oort et al., 2006; Levine et al., 1994). Canines also exhibit circadian and diurnal fluctuations in melatonin levels (Stankov et al., 1994). Average blood levels of melatonin are in the nanomolar range. The concentration of melatonin can fluctuate by two-fold during seasonal light cycle changes (Levine et al., 1994). Older individuals with lower melatonin levels may be more susceptible to neurodegeneration caused by oxidative stress (Clapp-Lilly et al., 2001). The northern location of Fairbanks, Alaska is at 65°N, and experiences anywhere from 21 hours of daylight in the summer to 4 hours in the winter. This dramatic change is expected to affect the body's internal clock by dictating the amount of melatonin produced.

Though melatonin has been shown to respond to changes in light, the exact physiological role of melatonin is uncertain. In many mammals it is well established that the timing and duration of melatonin synthesis is responsible for seasonal patterns in breeding, coat shedding, and color changes (Reiter and Robinson, 1995). It was previously believed that light variation was solely responsible for these changes, but temperature may affect melatonin production too. When Djungarian hamsters were exposed to cold temperatures the pineal gland was reported to have a reduced sensitivity to light, preventing the inactivation of N-acetyltransferase, the enzyme that converts serotonin to melatonin (Stieglitz et al., 1991). Similar results linking melatonin to thermoregulation have been reported in rats, newborn seals, and Siberian hamsters (Larkin et al., 2001; Stokkan et al., 1995; Tannenbaum, 1988). Reierth et al. (1999) reported nearly undetectable melatonin levels in Svalbard ptarmigan throughout the summer months and reduced amplitude and production in midwinter. They attributed these findings to a possible adaptation to life in the Arctic. Based on these findings, it could be assumed that while light may be the primary factor influencing melatonin secretion, some adapted animals have established a defensive mechanism to regulate hormonal levels as the seasons change (i.e. seasonal adaptation; Nelson et al, 2002).

The timing and amplitude of melatonin production has been implicated in seasonal affective disorder (SAD) (Danilenko et al., 1994; Nelson et al., 2002). Light therapy has been shown to alleviate symptoms of depression in the winter (Danilenko et al., 1994; Leppamaki et al., 2002). Exercise alone or in combination with light therapy has been reported to have a positive impact on seasonal mood slumps (Leppamaki et al., 2002). Sled dogs raised and housed in Alaska are exposed to the extreme light cycles and temperature ranges of the Arctic. A diurnal pattern was observed in sled dogs living in New York and Alaska with exercise being an additional influence on melatonin production in the current study. Exercise and adaptation to the environment may have an effect on melatonin levels in the circumpolar north, and sled dogs have been shown to be a unique model for illustrating effects on the endocrine and immune systems (Felsburg, 2002).

As the circumpolar north becomes more populated, understanding and developing models of normal hormonal secretion patterns in the general population takes on significance in regard to depression, sleep cycle and altered behavioral patterns. The dog has been an important medical research model because canines develop many of the same chronic diseases as humans (Adams et al., 2000; Kearns et al., 1999). For the circumpolar north, racing sled dogs are excellent models for studying health effects related to exercise and nutrition because they share the same environment, including exposure to same climate and light cycles. Additionally, much of their biochemical and endocrine mechanisms are similar to humans (Felsburg, 2002), yet their basal metabolic rate and energy expenditure is 3-8 times greater (Hinchcliff et al., 1997). An increased knowledge of normal variations in seasonal fluctuations will provide further understanding of adaptation to the environmental extremes. Also, exercise research has shown many benefits (Kell et al., 2001) and sled dogs have provided a good model system for studying exercise related biochemical changes (Dunlap et al., 2006; Reynolds et al., 1999).

2.3 Material and Methods

2.3.1 Animals

Alaskan huskies, *Canis lupis familiaris*, raised in Fairbanks, Alaska (Latitude, 65°N) or North Creek, New York (Latitude 45°) were used as test subjects. The Institutional Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#03-45). The dogs that were used in this study were typical racing sled dogs owned by Arleigh Reynolds or Gerald Mulvey, respectively. Twenty four sled dogs in Alaska, designated as the study dogs, were separated into 2 equal groups of 12, balanced for age, sex and ability. Similarly, 19 dogs in NY were separated by the kennel owners into 2 groups. The 2 groups were, 7 dogs in control (CON) and 12 exercise (EX) dogs. The average age of the dogs in AK was 3.9 with 55% males. The average age of the dogs in NY was 3.6 with 65% males. One dog was eliminated from the NY CON group prior to the termination of the study due to a diagnosis of lymphoma. Housing arrangements consisted of 2.5-m chains on which the dogs were tethered for the duration of the study (6 months). Each dog had access to his or her own house, and exposed to seasonal light and temperature conditions.

2.3.2 Diet

To insure that the dogs were acclimated to the diets, they were maintained on the study diet for 2 months preceding the study. A measured amount of food (approximately 450 g/day) was fed to each dog. The amount varied slightly throughout the study for each dog in order to maintain ideal body condition. Ideal body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum (Laflamme, 1997; Reynolds et al., 1999). During the acclimation period, the dogs were fed once a day in the morning. During the actual experiment the dogs were fed 12 hours prior to blood collection to insure that the dogs were in a post-absorptive state.

2.3.3 Exercise

All exercising sled dogs were in a training program developed by the kennel owner. Although training programs varied depending on the individual kennel, sled dogs exercise regimes are similar and focus on events that take place during the winter and spring. CON dogs were not involved in any formal exercise program throughout the duration of the study. Typically sled dogs do not compete in events in the summer and autumn, and this was the case for both kennels. Informal exercise was administered in the summer, including free running and swimming. Training for the winter begins in September. All dogs involved were sprint type sled dogs that compete in weekend races that vary in distance from 8 to 20 miles per day and average 20 miles per hour. Both teams are highly competitive both regionally and nationally.

2.3.4 Blood Sampling

All dogs were bled on the winter and summer solstices. On each of the solstice's blood was drawn at 2:00, 8:00, 10:30 and 17:00. These times were chosen based on previously reported variation in melatonin levels (Levine et al., 1994). Blood was drawn by venipuncture from the jugular into three 5 ml vacutainer tubes. Serum was obtained by centrifugation at 2500 x g for 10 min, transferred into freezer vials, flash frozen in liquid nitrogen and stored at –70°C until they were analyzed.

2.3.5 Clinical Analysis

All samples were sent to the Morehouse School of Medicine for analysis via standard radioimmunoassay. Melatonin was extracted from the serum (50 μL) using chloroform and then melatonin levels were measured by radioimmunoassay using a commercially available kit (ALPCO Diagnostics, Salem, NH). The sensitivity of the assay was 0.2 pg/ml. Intra-Assay variability was 9% and the inter-Assay was 13% (see Fukuhara et al., 2005 for more details).

2.3.6 Statistics

Results were determined using repeated measures analysis of variance (PROC MIXED IN SAS). The dogs were nested within region and type of exercise. There were repeated measurements on the same dogs for the 2 seasons and 4 sampling times. Due to the double repeated measures, seasons and sampling times, a 'direct product' covariance structure was used with an unstructured covariance matrix for the season and a compound symmetry covariance matrix for the hours sampled.

Statistical significance in inter-seasonal and diurnal variation for canines living in both New York (45° N) and Alaska (65° N) was determined with a $p \leq 0.05$. The figures do not show statistical differences for clarity but selected means are displayed in Table I.

2.4 Results

Sled dogs living at both lower and higher latitudes displayed peak melatonin levels in the winter that were significantly higher than summer values (Figure 2.1, Figure 2.2, Table 2.1). Figure 2.1 represents the daily and seasonal fluctuations in plasma melatonin concentrations of exercising and non-exercising sled dogs living at 65° N. Statistical comparisons were made across time, group and season. This is consistent with the typical diurnal rhythm of melatonin in mammals. The diurnal pattern in the summer at both latitudes was also typical with maximal secretion around 2:00 followed by a sharp decline at 8:00 and depressed levels throughout the daytime hours (Figure 2.1, Figure 2.2, Table 2.1). Sled dogs living at the lower latitude in the winter experienced a similar diurnal pattern as in the summer. In contrast, the peak in melatonin secretion at higher latitude in the winter for both exercise and non-exercise dogs was prolonged (Figure 2.1). Serum melatonin levels did not differ significantly between 2:00 and 8:00 in sled dogs in Alaska, and these sampling points were significantly higher than those at 10:30 and 17:00, suggesting extended duration of melatonin production (Figure 2.1, Table 2.1).

Figure 2.1 compares the mean serum levels between exercising and non-exercising sled dogs raised in New York. The seasonal summer pattern in melatonin production is similar in both lower latitude and higher latitude sled dogs but interestingly, overall and seasonal melatonin levels were significantly higher at lower latitude, than at higher latitude (Table 2.1). NY winter sled dogs displayed a typical trend in peak melatonin levels at 2:00 with a decline observed by 8:00 (Figure 2.1), unlike winter Alaskan sled dogs (Figure 2.1, Table 2.1).

Exercise significantly reduced melatonin levels in both the winter and summer in Fairbanks, AK (Figure 2.1). In New York, exercise had a pronounced effect on melatonin secretion in the winter but did not have an effect in the summer. Exercise appears to reduce winter melatonin levels to values comparable to summer values (Figure 2.1, Figure 2.2).

2.5 Discussion

At higher latitudes, the seasonal pattern of light and day length varies between 21 hours of daylight in the summer and 4 hours in the winter. Outdoor luminosity is also reduced because of the angle of the sun. In mammals, a specialized region of the brain, the suprachiasmatic nucleus, functions as the master pacemaker of circadian and diurnal rhythms and regulates melatonin secretion in the pineal gland. In this naturalistic study, young, healthy sled dog populations were compared to observe the effect of latitude and light cycle on endogenous melatonin production. The findings of seasonal fluctuations in melatonin, including an extended duration in production in the winter, were similar to an earlier study on seasonal secretion patterns in humans (Levine et al., 1994). At higher latitudes in the winter, regardless of exercise, there was no significant difference in melatonin levels between 2:00 and 8:00 as compared to both Alaska summer and the lower latitude melatonin levels. This suggests that in the winter in the Arctic there is a longer duration of melatonin secretion and/or the peak in melatonin production is shifted to the right. Additional sampling is necessary to determine which scenario is likely.

In spite of the longer duration of secretion, winter melatonin values at higher latitude were significantly lower than winter levels at the lower latitude. This supports the observation by Stieglitz et al. (1991) that showed that the pineal gland in hamsters exposed to cold temperatures had a reduced sensitivity to light, causing the inactivation of N-acetyltransferase. Similar reports connecting thermoregulation to melatonin secretions were reported in rats (Tannenbaum et al., 1988), seals (Stokkan et al., 1995), and Siberian hamsters (Larken et al., 2001), suggesting mammals living in Arctic and sub Arctic climates may have the ability to adapt to colder environments. Sled dogs in Fairbanks, AK are frequently exposed to extreme winter temperatures (-20 to -40°C) and the reduced melatonin levels observed in Alaska, as compared to their lower latitude counterparts, suggests their seasonal adaptation to the severe temperatures of the Arctic and sub Arctic.

The factor showing the most pronounced reductions in plasma melatonin levels in the current study was exercise. This observation may be translated to the clinic in that the timing and amplitude of melatonin secretion has been linked with seasonal affective disorder (SAD), described as depressive episodes recurring regularly during the winter season (Danilenko et al., 1994). A common treatment for symptoms of depression associated with SAD is light therapy (Danilenko et al., 1994; Leppamaki et al., 2002), and Leppamaki et al. (2002) showed that exercise alone, or in combination with light therapy, also alleviates symptoms of SAD in humans. The feedback mechanism involved in melatonin suppression by exercise treatment is still unknown but Leppamaki et al.

(2004) postulates that physiologically exercise may induce changes in central neurotransmitter concentrations.

Our results show a significant decrease in melatonin production in sled dogs with exercise decreasing melatonin secretion to levels comparable to summer melatonin values. Exercising sled dogs at the lower latitude also experienced a decrease in melatonin production, but this affect was not observed in the summer months. This may be due to slight variations in exercise protocol, especially in the summer. Sled dog competitions start at the earliest in late November and extend through the beginning of April. Both kennels trained for weekend events within this time frame, so exercise regimes in the winter would be similar, alleviating differences in fitness, but not in the summer. Based on our findings, exercise has a prominent effect on melatonin secretion, and this suggests that, similar to humans, exercise will have a positive impact on mood and performance in sled dogs.

Since melatonin has significant effects on sleep and man's adapting to the circumpolar north (Levine et al., 1994), sled dog studies should enhance our understanding of how exercise and diet can impact behavior in climates with extreme temperatures and light variations. In the present report, winter sampling was done very close to the solstice, insuring minimal exposure to natural light when sampling. To develop a complete profile of melatonin, especially around the winter solstice, diurnal sampling would need to be performed at smaller and more frequent intervals. Our data suggests a shift in the time of peak melatonin production in arctic regions, but without a definite serum melatonin value between 2:00 and 8:00, it is unclear whether the peak lies between these two points or that the peak in melatonin production is in proportion to the night length. Although the functional relevance of serum melatonin in circulation is unclear, melatonin production is thought to impact various body systems, especially the immune system (Nelson et al., 2002). These findings should lead to future studies focusing on the mechanisms of interaction between systems in seasonally adapted mammals.

2.6 Acknowledgements

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2.7 References

Adams, B., Chan, A., Callahan, H., Siwak, C., Tapp, D., Ikeda-Douglas, C., Atkinson, P., Head, E., Cotman, C.W. and Milgram, N.W. (2000) Use of a delayed non-matching to position task to model age-dependent cognitive decline in the dog. *Behav Brain Res*, **108**, 47-56.

Clapp-Lilly, K.L., Smith, M.A,, Perry, G., Harris, P.L., Zhu, X., and Duffy, L.K. (2001) Melatonin acts as anti-oxidant and pro-oxidant in an organotypic slice culture model of Alzheimer's disease. *Neuroreport*, **12**, 1277-80.

Danilenko, K.V., Putilov, A.A., Russkikh, G.S., Duffy, L.K., and Ebbesson, S.O. (1994) Diurnal and seasonal variations of melatonin and serotonin in women with seasonal affective disorder. *Arct Med Res*, **53**, 137-45.

Dunlap, K.L., Reynolds, A.J., and Duffy, L.K. (2006) Total antioxidant power in sled dogs supplemented with blueberries and the comparison of blood parameters associated with exercise. *Comp Biochem Physiol A Mol Integr Physiol*, **143**(4), 429-34.

Felsburg, P.J. (2002) Overview of immune system development in the dog: comparison with humans. *Human Exp Toxicol*, **21**, 487-92

Fukuhara, C., Aguzzi, J., Bullock, N.M., and Tosini G. (2005) Effect of long-term exposure to constant dim light on the circadian system of rats. *Neurosignals*, **14**, 117-25.

Hinchcliff, K.W., Reinhart, G.A., Burr, J.R., Schreier, C.J. and Swenson, R.A. (1997)

Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold. *Am J Vet Res*, **58**, 1457-1462.

Kearns, R.J., Hayek, M.G., Turek, J.J., Meydani, M., Burr, J.R., Greene, R.J., Marshall, C.A., Adams, S.M., Borgert, R.C. and Reinhart, G.A. (1999) Effect of age, breed and dietary omega-6 (n-6): omega-3 (n-3) fatty acid ratio on immune function, eicosanoid production, and lipid peroxidation in young and aged dogs. *Vet Immunol Immunopathol*, **69**, 165-183.

Kell, R.T., Bell, G., and Quinney, A. (2001) Musculoskeletal fitness, health outcomes and quality of life. *Sports Med*, **31**, 863-873.

Laflamme, D. (1997) Development and Validation of a body Condition Score System for Dogs. *Canine Practice*, **22**, 10-15.

Larkin, J.E., Freeman, D.A., and Zucker, I. (2001) Low ambient temperature accelerates short-day responses in Siberian hamsters by altering responsiveness to melatonin. *J Biol Rhythms*, **16**(1), 76-86.

Leppamaki, S.J., Partonen, T.T., Hurme, J., Haukka, J.K., and Lonnqvist, J.K. (2002) Randomized trial of the efficacy of bright-light exposure and aerobic exercise on depressive symptoms and serum lipids. *J Clin Psychiatry*, **63**(4), 316-21.

Leppamaki, S., Haukka J., Lonnqvist, J. and Partonen, T. (2004) Drop-out and mood improvement: a randomized controlled trial with light exposure and physical exercise. *BMC Psychiatry*, **4**, 22.

Levine, M.E., Milliron, A.N., and Duffy, L.K. (1994) Diurnal and seasonal rhythms of melatonin, cortisol and testosterone in interior Alaska. *Arct Med Res*, **53**, 137-145.

Lincoln, G.A. (2006) Decoding the nightly melatonin signal through circadian clockwork. *Mol Cell Endocrinol*, **252**, 69-73.

Nelson, R.J., Demas, G.E., Klein, S.L., and Kriegsfeld, L.J. (2002) Seasonal patterns of stress, immune function, and disease. Cambridge University Press, Cambridge, United Kingdom.

Reynolds, A.J., Reinhart, G.A., Carey, D.P., Simmerman, D.A., Frank, D.A., and Kallfelz, F.A. (1999) Effect of protein intake during training on biochemical and performance variables in sled dogs. *Am J Vet Res*, **60**(7), 789-95.

Rajaratnam, S.M., Dijk, D.J., Middleton, B., Stone, B.M., and Arendt, J. (2006) Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *J Clin Endocrinol Metab*, **88**(9), 4303-9.

Reierth, E., Van't Hof, T.J., and Stokkan, K.A. (1999) Seasonal and daily variation in plasma melatonin in the high-arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *J Biol Rhythms*, **14**(4), 314-9.

Reiter, R.J., and Robinson, J. (1995) Melatonin. New York, Bantam Books.

Stankov, B., Moller, M., Lucini, V., Capsoni, S., and Fraschini, F. (1994) A carnivore species (*Canis familiaris*) expresses circadian melatonin rhythm in the peripheral blood and melatonin receptors in the brain. *Eur J Endocrinol*, **131**(2), 191-200

Stieglitz, A., Steinlechner, S., Ruf, T., and Heidmaier, G. (1991) Cold prevents the light induced inactivation of pineal N-acetyltransferase in the Djungarian hamster, *Phodopus sungorus*. *J CompPhysiol A*, **168**, 599-603.

Stokkan, K.A., Vaughan, M.K., Reiter, R.J., Folkow, L.P., Martensson, P.E., Sager, G., Lydersen, C., and Blix, A.S. (1995) Pineal and thyroid functions in newborn seals. *Gen Compar Endocrin*, **98**, 321-331.

Tannenbaum, M.G., Reiter, R.J., Vaughan, M.K., Troiani, M.E., and Gonzalez-Brito, A. (1988) Effects of short-term cold exposure on pineal biosynthetic function in rats. *Cryobiology*, **25**, 227-32.

Van Oort, B.E., Tyler, N.J., Gerkema, M.P., Folkow, L., and Stokkan, K.A. (2006) Where clocks are redundant: weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften*, **94**(3), 183-94.

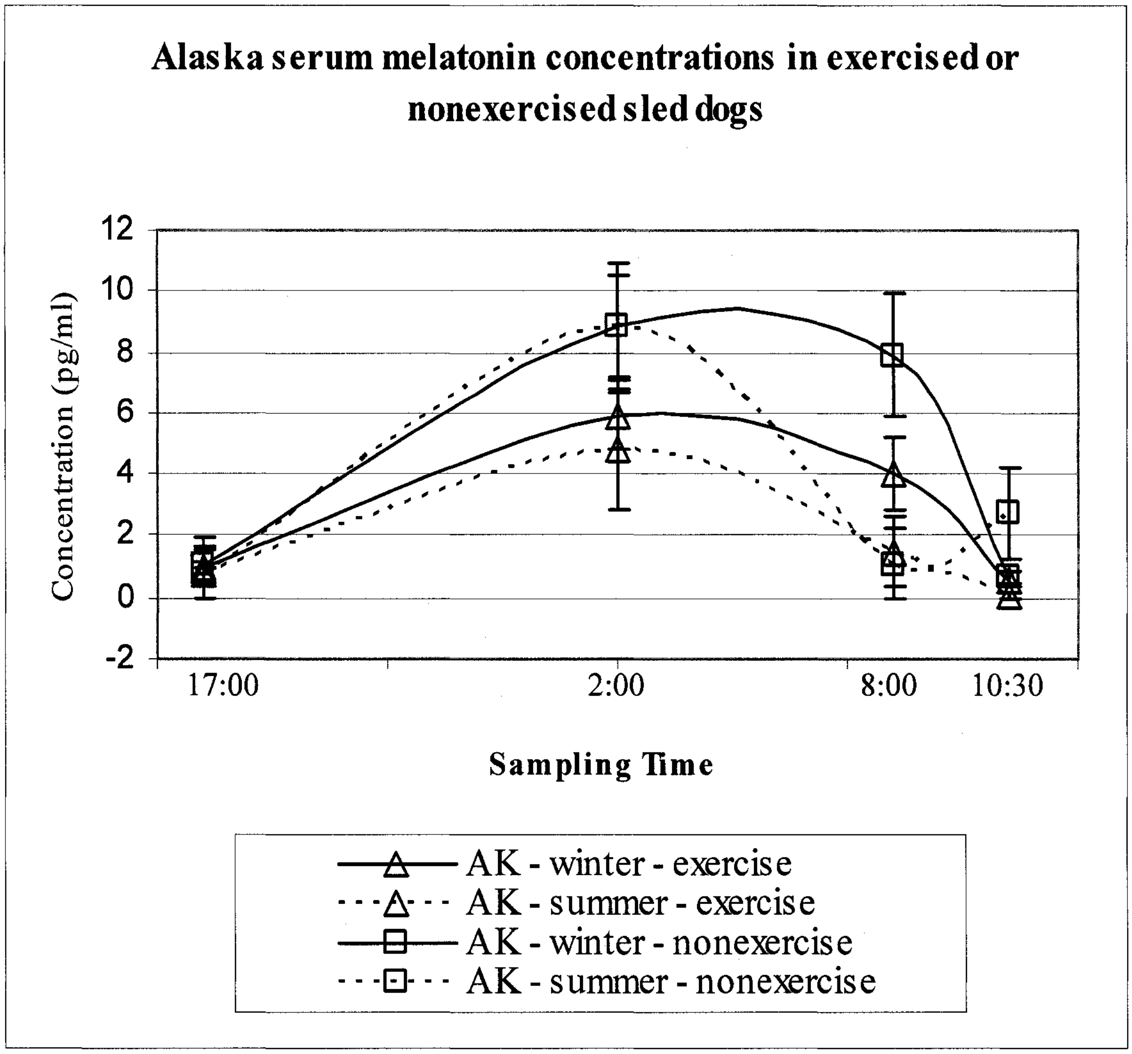

Figure 2.1: The diurnal pattern of melatonin in the serum of sled dogs in Fairbanks, AK (65°N). Samples were taken from both exercising and non-exercising sled dogs near the winter and summer solstices.

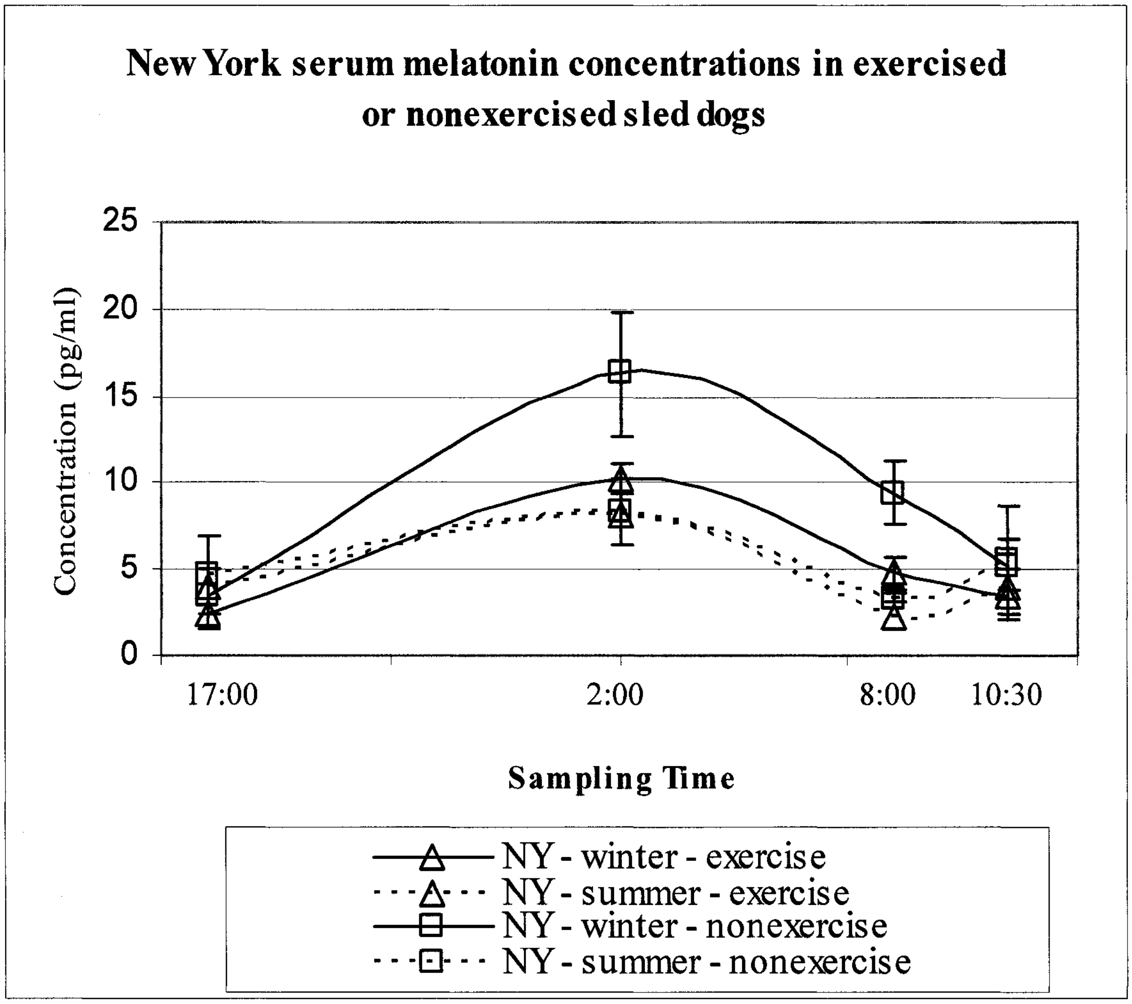

Figure 2.2: The diurnal pattern of melatonin in the serum of sled dogs in North Creek, NY (45°N). Samples were taken from both exercising and non-exercising sled dogs near the winter and summer solstices.

Table 2.1: Selected means and SEM in serum melatonin levels of sled dogs across latitude, season, group and time. Significance is established with $p \leq 0.05$. Significance within a row is shown with an asterisks (*). For clarity, since there are many possibilities for comparisons, not all significant relationships are shown. Significance across latitude, time and group is shown with letter superscripts. Capital letter superscripts are compared with the same letter in lower case superscripts and have a significant relationship.

NY (all)	2.39±0.18*	**AK (all)**	1.26±0.18*
Winter Exercise	1.63±0.20*	**Winter Non-exercise**	2.64±0.24*
Summer Exercise	1.28±0.23	**Summer Non-exercise**	1.76±0.27
Summer (all)	1.52±0.18*	**Winter (all)**	2.13±0.15*
NY Winter (all)	2.77±0.23*	**AK Winter (all)**	1.50±0.20*
17:00	1.16±0.40[a,b]	17:00	0.41±0.35[c]
2:00	5.29±0.40[A]	2:00	2.95±0.35[C,a]
8:00	2.84±0.40[a,B]	8:00	2.38±0.35[C]
10:30	1.76±0.40[a,b,D]	10:30	0.24±0.36[c,d]
NY Summer (all)	2.01±0.27*	**AK Summer (all)**	1.02±0.23*
17:00	1.72±0.47[H,e]	17:00	0.30±0.40[f,h]
2:00	3.28±0.46[E,a]	2:00	2.72±0.40[F]
8:00	1.14±0.48[b,e]	8:00	0.52±0.40[c,f]
10:30	1.91±0.47[G,e]	10:30	0.55±0.40[f,g]

Chapter 3

Cross-latitudinal, seasonal and diurnal comparisons in thyroid hormone concentrations in sled dogs*

3.1 Abstract

Objective— To investigate the effects of light exposure, climate, latitude, exercise, and season on serum triiodothyronine (T_3), free triiodothyronine (FT3), thyroxine (T_4), free thyroxine (FT4) and thyroid stimulating hormone (cTSH) concentrations in sled dogs. With this data, establish appropriate reference levels for racing sled dogs and establish a better understanding of environmental impacts on thyroid function.

Design— Cross-latitudinal naturalistic study.

Animals— 43 sled dogs.

Procedure— Diurnal serum thyroid hormone concentrations were measured on the winter and summer solstices in exercising and non-exercising sled dogs, located in North Creek, NY (45°N) and Fairbanks, AK (65°N).

Results— We observed diurnal variations in serum thyroid hormone levels that depended on season, latitude, day length and to a lesser extent exercise. In the winter, non-exercising sled dogs had higher cTSH levels than exercising dogs, regardless of latitude. TT3 and FT3 were elevated during the winter solstice and this was much more

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pronounced in Alaskan sled dogs. There was no observed diurnal variation in FT4 and TT4 levels in Alaskan sled dogs on the winter solstice when there was only 3.5 hours of low light but a definite diurnal variation in these same dogs in the summer when there was 21 hour of daylight.

Conclusions and Clinical Relevance— Elevated T_3 levels in sled dogs in the winter, with a more pronounced difference in Alaska, suggests an effect of thermoregulation or day length on thyroid function. Exercise may have temporarily suppressed thyroid function, but this effect did not extend throughout the day. Standardizing sampling time would improve comparative studies and advance the field. Our results agree with previous reports suggesting a lowered reference range for thyroid hormone concentrations in sled dogs.

3.2 Introduction

Thyroid hormone production is regulated by the hypothalamic-pituitary-thyroid hormone negative-feedback axis.[1] The main form of thyroid hormone produced by the thyroid gland is thyroxine (T_4). Extrathyroid tissues regulate the deiodization of the "prohormone" T_4 to the active form of the hormone, triiodothyronine (T_3). Small increases in T_4 and T_3 suppress the production and secretion of thyroid stimulating hormone (cTSH) through alterations in nuclear receptor binding, mRNA transcription and protein synthesis. Thyroid hormones in serum are either in a free form or are bound to carrier proteins such as thyroid hormone binding globulin (TBG), transthyretin, albumin and apolipoproteins. Small concentrations of free hormone are in a dynamic equilibrium with thyroid hormone bound to these carrier proteins. Both free and bound thyroid hormone reversibly enters interstitial fluid from circulating blood in a tissue dependant manner. Free hormone subsequently moves into the cell and either binds to cytosol-binding proteins and nuclear receptors, or is metabolized. Previous studies have shown that dogs have a higher concentration of free T_4 than humans and a lower binding affinity between T_4 and binding proteins. In addition, dogs, when compared to humans, exhibit a wider range of daily fluctuations in serum T_4.[2,3]

Hypothyroidism is the most common endocrine disorder in dogs, often caused by autoimmune destruction or idiopathic atrophy of the thyroid gland. Replacement therapy involves oral administration of exogenous L-thyroxine.[2] Despite this relatively easy solution to a common problem, the complexity of the thyroid hormone regulation and feed back systems can lead to complications. Hypothyroidism affects many organ systems making it difficult to diagnose upon initial inspection. Compounding this diagnostic ambiguity on non-specific abnormalities is the uncertainty derived from canine T_4 test results.[4] Euthyroid dogs can often have low or borderline T_4 levels because of normal fluctuations in serum T_4, age, breed variations, other illnesses, or interfering medications.[4]

Documented deviations in human thyroid hormones have resulted from seasonal changes,[5,6] exercise,[7] diurnal variations[3,8] and possibly as a consequence of light.[5] Cold environments are known to heighten thyroid activity,[6] but the mechanism is uncertain. Fluctuations in thyroid hormone are not limited to seasonal cycles but a diurnal cycle is also present. In humans, serum T_4 concentrations reach a high between 10:00 AM and 2:00 PM, and a low around 2:00 AM. A reverse trend is seen for cTSH. In contrast, dogs exhibit more random variations in thyroid hormone throughout the day due to decreased protein binding affinity and half-life differences.[3] Hoh and Oh did not report any diurnal variation in T_3 but found that Total T_4 (TT4) and Free T_4 (FT4) peaked between 11:00 and 14:00.[9] Bruner et al. reported no diurnal variations in cTSH in hypothyroid and euthyroid dogs, collected over a 12 hour sample period.[10]

Sled dogs sampled before and after competing in a long distance race were sampled for T_3, T_4, total protein, and albumin concentrations which all decreased significantly from pre-race to post-race. Conditioned sled dogs often display below the normal reference range for thyroid hormones.[7,11] Other breeds, such as sight hounds, also have lower than normal serum thyroid hormone concentrations.[2] Based on all the variables associated with racing sled dogs and reported thyroid hormone levels, Lee et al. suggested lowering the reference ranges for the sled dog.[11] In this study, we address the question of whether or not sled dogs exposed to more extreme daylight and temperature are more susceptible to hypothyroidism as judged by hormone levels. We present data from a cross-latitudinal study, looking at both exercising and non-exercising sled dogs.

3.3 Material and Methods

3.3.1 Animals

Alaskan huskies, *Canis lupis familiaris*, raised in Fairbanks, Alaska (Latitude, 65°N) or North Creek, New York (Latitude 45°) were used as test subjects. The Institutional Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#03-45). The dogs that were used in this study were privately owned typical Alaskan husky sled dogs. Nineteen dogs in NY, designated as the study dogs, were separated by the kennel owners into 2 groups, balanced for age, sex and ability. In New York there were 7 control (CON) and 12 exercise (EX) dogs. Similarly 24 sled dogs in Alaska were separated into 2 equal groups of 12. One dog was eliminated from the NY CON group prior to the termination of the study due to a diagnosis with lymphoma. The average age of the dogs in AK was 3.9 with 55% males. The average age of the dogs in NY was 3.6 with 65% males. Housing arrangements consisted of 2-m chains on which the dogs were tethered for the duration of the study (6 months). Each dog had access to his or her own house.

3.3.2 Diet

To insure that the dogs were acclimated to the diet, they were maintained on the study diet for 2 months preceding the study. All dogs were fed a measured amount of Purina Pro Plan Performance® daily (approximately 450 g). The amount varied slightly throughout the study for each dog in order to maintain ideal body condition. Ideal body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum.[12,13] During the acclimation period, the dogs were fed once a day in the morning. During the actual experiment the dogs were fed 12 hours prior to blood collection to insure that the dogs were in a post-absorptive state.

3.3.3 Exercise

All exercising sled dogs were in a training program developed by the kennel owner. Although training programs varied depending on the individual kennel, the exercise regimes were similar and focused on events that took place during the winter and spring. No exercise was performed at least 12 hours prior to sampling. CON dogs were not involved in any formal exercise program throughout the duration of the study.

3.3.4 Blood Sampling

All dogs were bled on the winter and summer solstices. Average ambient temperature in New York on the summer sampling date was 18.7°C and -11.3°C in the winter. Likewise, average ambient temperature in Alaska on the summer sampling date was 23.0°C and -13.6°C in the winter. On each of the solstice's blood was drawn at 2:00, 8:00, 10:30 and 17:00. At the equinox blood was drawn at 17:00. These times were chosen based on reported fluxes in thyroid hormones.[3,8] Blood was drawn by venipuncture from the jugular into three 5 ml vacutainer tubes containing no anticoagulant. Serum was obtained by centrifugation at 2500 X g for 10 min, transferred into freezer vials, flash frozen in liquid nitrogen and stored at –70°C until they were analyzed.

3.3.5 Endocrine Assays

Endocrine assays for this study were performed at the Endocrine Section, Diagnostic Center for Population and Animal Health, Michigan State University. Commercially available radioimmunoassay kits, validated for use in canine serum, were utilized for assay of total thyroxine (TT4),[14] free thyroxine by equilibrium dialysis (FT4),[15] and free triiodothyronine (FT3).[16] Serum concentrations of total triiodothyronine (TT3) were measured using an in-house charcoal-separation radioimmunoassay, of which the procedure[17] and validation for dogs[18] were previously reported. Thyroid stimulating hormone (cTSH) was measured with a commercially available immunoradiometric assay kit, with previously reported performance data for the laboratory.[14]

3.3.6 Statistics

Data was analyzed using repeated measures analysis of variance.[a] The dogs were nested within region and type of exercise. There were repeated measurements on the same dogs for the 2 seasons and 4 sampling times. Due to the double repeated measures, seasons and sampling times, a 'direct product' covariance structure was used with an unstructured covariance matrix for the season and a compound symmetry covariance matrix for the hours sampled.

3.4 Results

3.4.1 Free T_4 (FT4)

Exercising sled dogs did not have significantly different FT4 levels than their non-exercising counterparts at either latitude (data not shown). New York serum FT4 levels were significantly lower in the summer at 17:00 than FT4 levels in Alaska sled dogs. In the winter New York sled dogs had significantly lower FT4 levels than Alaska dogs at 2:00. There are opposite diurnal trends in the winter (increasing then decreasing) verses the summer (decreasing then increasing) in serum FT4 levels in Alaska sled dogs, with significant differences at 2:00 (Figure 3.1). In the winter Alaska dogs experience peak levels at 2:00, while the low for these dogs in summer is at 2:00. In New York dogs, serum FT4 levels differ at 17:00 and 10:30 between the seasons: with the peak at 10:30 and the low at 17:00 in the summer. Both peak and low levels were significantly different from winter values. The diurnal variations in New York dogs also differ with season. The diurnal variation in serum FT4 levels in Alaska sled dogs was very gradual and showed no significant differences between sampling times (Table 3.1). While for New York dogs, serum FT4 levels were significantly lower in the summer at 17:00 than all other time periods. At each consecutive sampling time there was a significant difference in serum FT4 that was higher than the previous sampling and lower than the next, starting from 17:00 and ending at 10:30. In contrast, in the winter, New York dog serum FT4 levels were significantly higher at 17:00 than at 2:00 and 10:30, where the levels at 8:00 were just slightly lower than at 17:00, but significantly higher than at 2:00 (Figure 3.1, Table 3.1).

3.4.2 Free T_3 (FT3)

As with FT4, exercise does not appear to affect FT3 levels (data not shown). In the summer serum FT3 levels did not significantly differ between Alaskan and New York dogs at any sampling time. While in the winter, New York dog serum FT3 levels were significantly higher than Alaska levels at 17:00 and 10:30. In addition, serum FT3 values peak at opposite times of the day in New York versus Alaska dogs; for Alaska the peak values are between 2:00 and 8:00 with significantly higher values than the other sampling times, where as in New York dogs the peak is between 10:30 and 17:00 with the significant low at 8:00. Within Alaska, sled dog summer serum FT3 levels were only significantly lower at 8:00 than at the same time in the winter. Again, the low level in the summer is at 8:00 while, in the winter, Alaska levels appear to be declining from their peak levels for the day at 8:00. The peak FT3 level in Alaska in the summer was at 2:00, with significant differences between all other time periods. In New York dogs, winter serum FT3 levels were significantly higher than summer levels at 17:00 and 10:30. The diurnal trend in the winter in New York appears to be opposite the New York summer trend, though there were no significant differences in FT3 between sampling time in the summer (Fig 3.2, Table 3.1).

3.4.3 Total T_4 (TT4)

Exercise did not significantly affect TT4 levels within season or latitude; however, TT4 levels were significantly higher at 17:00 than at 10:30 in exercising sled dogs, while the opposite was seen in the non exercise dogs (data not shown). In the summer, Alaskan sled dog's TT4 levels at 17:00 and 2:00 were significantly higher than sled dogs living in New York. Again, the diurnal trends in the summer for the different latitudes appear to be inversely related. Whereas 2:00 is the significant peak level for serum TT4 levels in Alaska dogs, with an increase beginning at 17:00, the opposite trend is seen in New York with significantly higher levels expressed between 8:00 and 10:30. With latitude, as well, the diurnal pattern for summer compared to winter also show opposite trends. In Alaska there is significant difference at 2:00 in the summer verses winter, while in the New York dogs, 8:00 and 10:30 levels in the summer are significantly higher than in the winter. Unlike FT4, serum TT4 levels in the winter do not differ by latitude, and New York and Alaska even follow the same trend with the significant peak observed at 17:00 (Fig 3.3, Table 3.1).

3.4.4 Total T_3 (TT3)

Exercise was not a major factor for determining serum TT3 levels (data not shown). Levels for TT3 in sled dogs were 1000 times higher than FT3 levels. The diurnal pattern in Alaska in the summer shows a low of serum TT3 levels at 8:00; with levels significantly lower than 17:00 and 2:00, the apparent peak of the day. In contrast, in New York dogs the serum TT3 levels peak between 8:00 and 10:30, with 8:00 levels significantly higher than 2:00 values and significantly higher than Alaska 8:00 values. Though there were no significant differences in the summer between Alaska and New York dogs for serum FT3, there is a similar overall trend with the peak level of the day in Alaska also at 2:00. The diurnal pattern in New York in the winter shows a more pronounced peak at 8:00 compared to the Alaska summer TT3 pattern. Very little diurnal variation exists for serum TT3 levels in Alaska dogs in the winter. Like the summer pattern, a slight peak exists at 2:00 with a significantly greater value than 10:30 level. Because such little diurnal variation is present, all values for Alaska are significantly higher than New York values, except at 8:00. Within latitude, all values for Alaska in the winter are significantly higher than all values in the summer. The difference is less exaggerated between winter serum TT3 levels in New York and summer than for Alaska, but again shows higher levels in winter, with significance at 17:00 and 8:00 (Fig 3.4, Table 3.1).

3.4.5 Thyroid stimulating hormone (cTSH)

Seasonality and exercise impacted cTSH production. In the summer, there was very little difference between exercise and non-exercise dogs, but in the winter, non-exercise dogs had significantly higher values, regardless of latitude (data not shown). The diurnal trend for serum cTSH in the summer for Alaska and New York sled dogs followed a similar pattern, with a significant peak at 17:00 (Figure 3.5). Although summer levels in Alaska appear to be higher at every sampling period, the only significant difference is at 10:30. In the winter New York dogs displayed no significant diurnal variation, and Alaskan and New York dogs significantly differed only at 8:00. Alaska dogs, on the other hand, displayed a diurnal pattern with a significant difference at 8:00 and a notable increase in cTSH at 10:30 which persisted throughout all other sampling times. The diurnal characteristics observed in Alaska, again, appear to follow opposite trends for the summer and winter, with the relative peak at 2:00 in the winter and the relative low peak being the same time in the summer. In New York dogs all winter values are significantly higher than summer values except for 17:00 where cTSH levels peak (Fig 3.5, Table 3.1).

3.5 Discussion

In all mammals, the thyroid gland is a central regulating gland, which regulates overall metabolic rate as well as stimulating cell and tissue growth. Due to the thyroid's role in body temperature regulation there are many studies examining the effects of cold temperatures on thyroid hormone levels. While some published reports show an increase in T_3 in winter months, others show a decrease of T_3 and T_4. It is difficult to generalize and the field remains unclear because of seasonal and diurnal factors. Our results support a more complicated picture than usually presented, with time of day being a major component in comparing thyroid hormone levels.

Availability of data on diurnal variation in dogs is limited, especially when factoring in variables such as day length. Other reports that the variation in T_3 and T_4 levels throughout the day is similar in dogs and humans, with the peak of T_3 and T_4 levels for humans reported between 10:00 and 14:00 [6] and peak of T_4 levels in dogs between 11:00 and 14:00.[9,10]

Interestingly, Alaskan dogs displayed no diurnal variation in serum FT4, and show an inverse relationship between the winter and summer (Fig 3.1). Serum FT4 levels in sled dogs at the lower latitude (New York) displayed apparent diurnal variations in both winter and summer. Without having sampling times between 10:30 and 17:00, it is difficult to compare our results with previous findings. In the summer in New York, sled dog's FT4 levels peaked at 10:30, close to the time previously reported.[9] However, in the winter New York dogs followed a different scenario, with dual peaks occurring at 17:00 and 8:00 (Fig 3.1). There were similar trends for serum TT4 in New York as there was for serum FT4. Interestingly, Alaska dogs had higher FT4 levels in the winter than summer, yet higher TT4 in the summer than winter (Fig 3.1, Fig 3.3, Table 3.1). Additionally, there was a significant diurnal variation in the summer for Alaska, with the peak occurring at 2:00 in the summer, and a trend for the opposite seen in the winter, yet this was not significant (Fig 3.3, Table 3.1). Serum TT4 levels in New York dogs, as with FT4 followed a different pattern than Alaska, with the summer peak and the winter low at 10:30 and winter peak and summer low 17:00 (Fig 3.1, Fig 3.3, Table 3.1). These results clearly illustrate an affect of day length on thyroid function, because in Fairbanks, Alaska at 2:00 a.m. on the summer solstice, it is still light, with the sun just rising. In the winter in Alaska, when there is only 3.5 hours of low light, there is no diurnal variation in FT4 and TT4 levels. Not only were there differences between New York and Alaska dogs, there was also different patterns displayed in the summer versus winter in the Alaskan dogs.

While Hoh and Oh did not report on T_3 levels in dogs,[9] our results support their observation of a diurnal pattern and indicate varying diurnal trends based on the factors of latitude (light) and season. Our results also differ from the reported pattern in humans, which displayed parallel trends for both T_3 and T_4.[5] In dogs, we observed separate trends for T_3 and T_4 and sometimes different trends for total verses free hormone levels. In the winter, New York and Alaska dogs display completely opposite patterns in FT3, with peaks and lulls at opposite times of the day. In the summer in New York dogs, there was not a significant diurnal trend yet overall levels in the summer were significantly lower than winter levels. Again the same dogs displayed different peaks and troughs depending on the season (Fig 3.2, Table 3.1). The opposite trend exists for TT3 and FT3 levels in the winter for New York, with 8:00 being the pivotal time for peak or lull. In contrast, the trend for TT3 for the summer values in New York resemble that of FT3 (Fig 3.2, Fig 3.4). There was very little diurnal variation in TT3 levels in Alaskan sled dogs in either winter or summer; the exception being a significant low at 8:00 in the summer as seen with FT3 (Fig 3.4, Table 3.1). However, serum TT3 levels in the winter, again tended to be higher than in the summer for all T_3 with significantly elevated levels at all time periods for Alaska winter, compared to summer (Fig 3.2, Fig 3.4, Table 3.1). The elevated T_3 levels may indicate an increase in energy expenditure to accommodate thermoregulation or may be caused by the change in daylight. As expected, the diurnal difference in dogs residing at higher latitude is more pronounced. However, sled dogs residing in Alaska do not appear to be more susceptible to hypothyroidism.

Bruner et al observed no diurnal variation in thyroid stimulation hormone secretion in euthyroid and hypothyroid dogs.[10] In the current study, serum cTSH levels in sled dogs living in New York in the winter also displayed no diurnal variation. However, this was the exception. The same dogs in New York in summer displayed significant peak cTSH levels at 17:00 and a lull at 2:00 (Fig 3.5, Table 3.1). In many cases the trends in cTSH were opposite from T_3 and T_4, as would be expected, but not in all cases.

Exercise did not appear to be a major influencer in FT3, TT3, or FT4 in this study. Total T_4 levels, however, were significantly higher at 17:00 than at 10:30 in exercising sled dogs, while the opposite was seen in the non-exercise dogs, regardless of season or location. Sled dogs are often exercised early in the day to avoid the heat. This reverse trend may indicate that exercise suppresses TT4 temporarily. Another indication that exercise may suppress thyroid function was the observation of lower TSH levels observed in the winter in exercise dogs compared with non-exercise dogs, regardless of latitude. Lee et al. and Panciera et al. reported reduced thyroid hormone production (TT4, FT4, and cTSH) at the completion of a long distance race that were compared with pre-race and off-season values.[11,18] The sled dogs participating in the above study were long distance, endurance sled dogs. The current study used sprint-type sled dogs that exercise at a higher intensity but for a much shorter duration. Additionally, these sled dogs were not sampled following an intense period of exercise, so the levels are more reflective of the basal concentrations in physically fit sled dogs. Any suppression of thyroid function or hormone production that occurred as a result of exercise, in the current study, was temporary and did not extend throughout the day.

Research indicates that thyroid function is affected by climate. Reed et al. described an increase in TSH, a decrease in total free T_3 and no change in total or free T_4 in human, euthyroid males during the first 42 weeks of the subjects' stay in Antarctica.[19] They have described this as the polar T_3 syndrome. Chengli et al. found similar results but a smaller difference in free T_3 and a decrease in total T_4.[20] Maes et al. reported a significant annual cycle in total T_3, with higher values in the winter and fall.[21] We also report higher levels T_3 in the winter than summer, akin to the results of Maes et al. Unlike the Antarctica studies, the dogs in this study were already acclimated to their environments. We examined Alaskan sled dogs that have been bred and raised in cold climates and experiencing similar stresses in order to reduce individual variability. This study showed sled dogs experience diurnal variation in thyroid hormones and this variation is affected to some extent by season, daylength, temperature, and exercise.

As clearly illustrated by the fluctuating thyroid hormone levels throughout the day, across season, and latitudes, diagnosing deficiencies in thyroid hormone production is not an easy task. Lee et al suggested that reference range for sled dogs may be lower than for other breeds.[11] The current reference range for thyroid hormones in dogs analyzed by the Animal Health Diagnostic Laboratory Endocrine Diagnostic Section at Michigan State University is 15-67 nmol/L for TT4, 6-42 pmol/L for FT4, 1-2.5 nmol/L for TT3, 4.5-12 pmol/L for FT3, and 0-37 mU/L for cTSH. The values reported in the present study agree strongly with the reference range suggested by Lee et al.[11] Our suggested reference ranges based on our means, medians and standard deviations are as follows: 7-31 nmol/L for TT4, 5-18 pmol/L for FT4, 0.8-1.6 nmol/L for TT3, 2.0-6.5 pmol/L for FT3, 5-17 mU/L for cTSH.

Lastly for diagnostic and research purposes we suggest that a fixed time of day for sampling be agreed upon by the veterinary community. Dogs are an excellent model for exercise research related to endocrinology and nutrition, and standardizing sampling time would improve comparative studies and advance the field.

3.6 Acknowledgements

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3.7 Footnotes

[a]PROC MIXED IN SAS, SAS Institute Inc., Cary, NC.

3.8 References

1. Nelson RJ, Demas GE, Klein SL, et al. Seasonal patterns of stress, immune function, and disease. Cambridge, United Kingdom: Cambridge University Press, 2002.
2. Ferguson DC. Update on diagnosis of canine hypothyroidism. *Vet Clin North Am Small Anim Prac* 1994; 24(3): 515-39.
3. Kaptein EM, Hays MT, Ferguson DC. Thyroid hormone metabolism: a comparative evaluation. *Vet Clin North Am Small Anim Prac* 1994; 24(3): 431-63.
4. Kemppainen RJ, Behrend EN. Diagnosis of canine hypothyroidism. *Vet Clin North Am Small Anim Prac* 2001; 31(5): 951-62.
5. Levine M, Duffy L, Moore DC et al. Acclimation of a non-indigenous sub-Arctic population: seasonal variation in thyroid function in interior Alaska. *Comp Biochem Physiol A* 1995; 111(2): 209-14.
6. Plasqui G, Kester ADM, Westerterp KR. Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. *Am J Physiol Endocrinol Metab* 2003; 285: E338-343.
7. Panciera DL, Hinchcliff KW, Olson J et al. Plasma thyroid hormone concentrations in dogs competing in long-distance sled dog race. *J Vet Intern Med* 2003; 17(4): 593-6.
8. Surks MI, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. *J Clin Endocrinol Metab* 2005; 90(9): 5489-96.

9. Hoh WP, Oh TH. Circadian variations of serum thyroxine, free thyroxine and 3,5,3'triiodothyronine concentrations in healthy dogs. *J Vet Sci* 2006; 7(1): 25-9.

10. Bruner JM, Scott-Moncrieff JC, Williams DA. Effect of time of sample collection on serum thyroid-stimulating hormone concentrations in euthyroid and hypothyroid dogs. *J Am Vet Med Assoc* 1998; 212(10): 1572-5.

11. Lee JA, Hinchcliff KW, Piercy RJ, et al. Effects of racing and nontraining on plasma thyroid hormone concentrations in sled dogs. *J AmVet Med Assoc* 2004; 224(2): 226-31.

12. Laflamme D. Development and Validation of a body Condition Score System for Dogs. *Canine Practice* 1997, 22, 10-15.

13. Reynolds AJ, Reinhart GA, Carey DP, et al. Effect of protein intake during training on biochemical and performance variables in sled dogs. *Am J Vet Res* 1999; 60: 789-795.

14. Paradis M, Sauve F, Charest J, et al. Effects of moderate to severe osteoarthritis on canine thyroid function. *Can Vet J* 2003; 44: 407-412.

15. Daminet S, Paradis M, Refsal KR, et al. Short term influence of prednisone and phenobarbital on thyroid function in euthyroid dogs. *Can Vet J* 1999; 40: 411-415.

16. Cerundolo R, Mauldin E, Goldschmidt M, et al. Adult-onset hair loss in Chesapeake Bay retrievers: a clinical and histological study. *Vet Derm* 2005; 16: 39-46.

17. Refsal KR, Nachreiner RF, Anderson CR. Relationship of season, herd, lactation, age and pregnancy with serum thyroxine and triiodothyronine in Holstein cows. *Dom Anim Endo* 1984; 3: 225-234.

18. Panciera DL, MacEwan EG, Atkins CE, et al. Thyroid function tests in euthyroid dogs treated with l-thyroxine. *Am J Vet Res* 1990, **51,** 22-26.

19. Reed LH, Burman KD, Mohamed Shakir KM, et al. Alterations in the hypothalamic pituitary-thyroid axis after prolonged residence in Antarctica. *Clin Endocrinol* 1986; 25: 55 65.

20. Chengli X, Guangjin Z, Quanfu X, et al. Effect of the Antarctic environment on hormone levels and mood of Chinese expeditioners. *Int J Circ Health* 2003; 62(3): 255-266.

21. Maes M, Mommen K, Hendrickz D, et al. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. *Clin Endocrin* 1997; 46: 587-598.

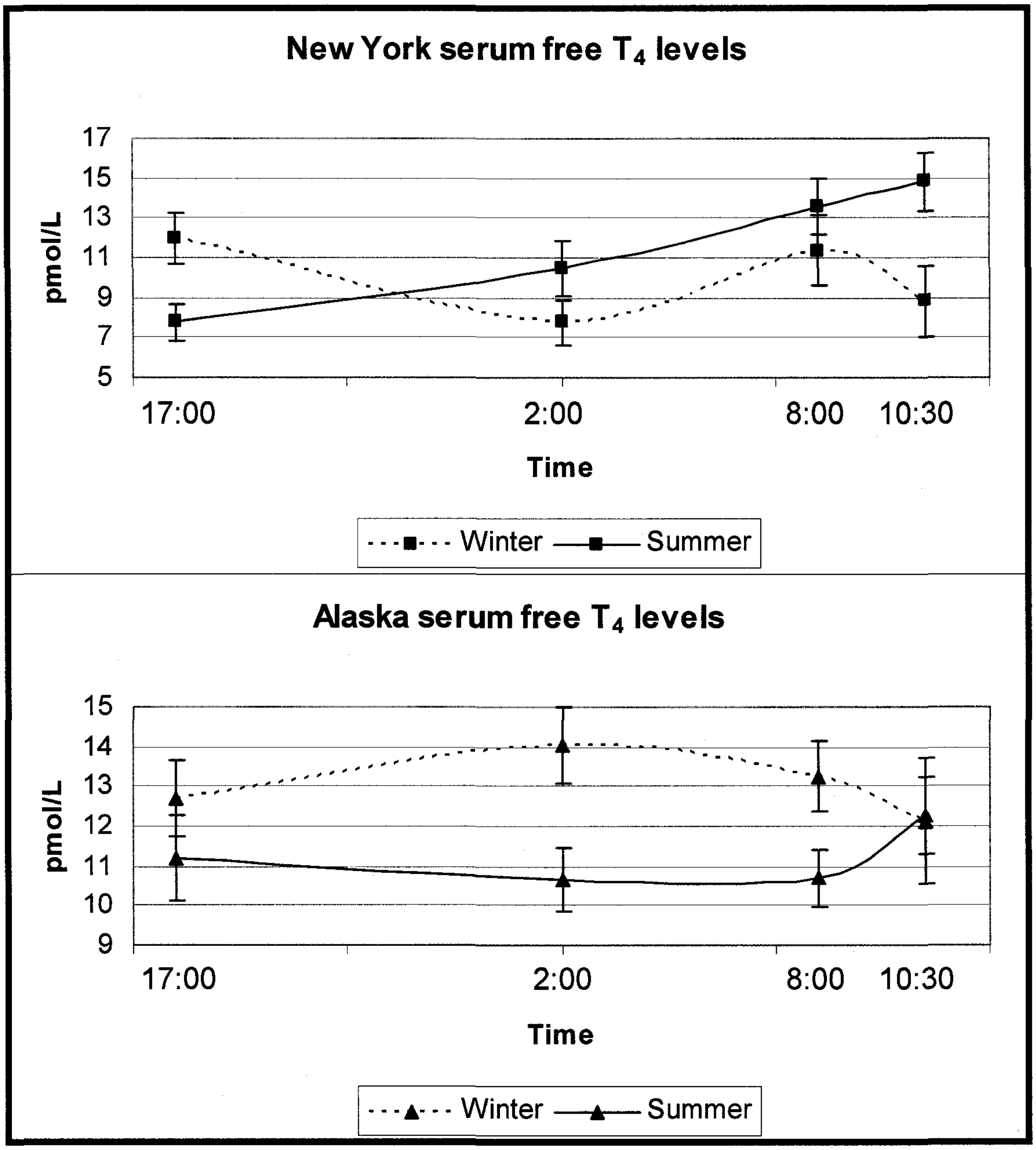

Figure 3.1: Serum free T_4 (FT4) in sled dogs living in New York or Alaska collected on the winter and summer solstice.

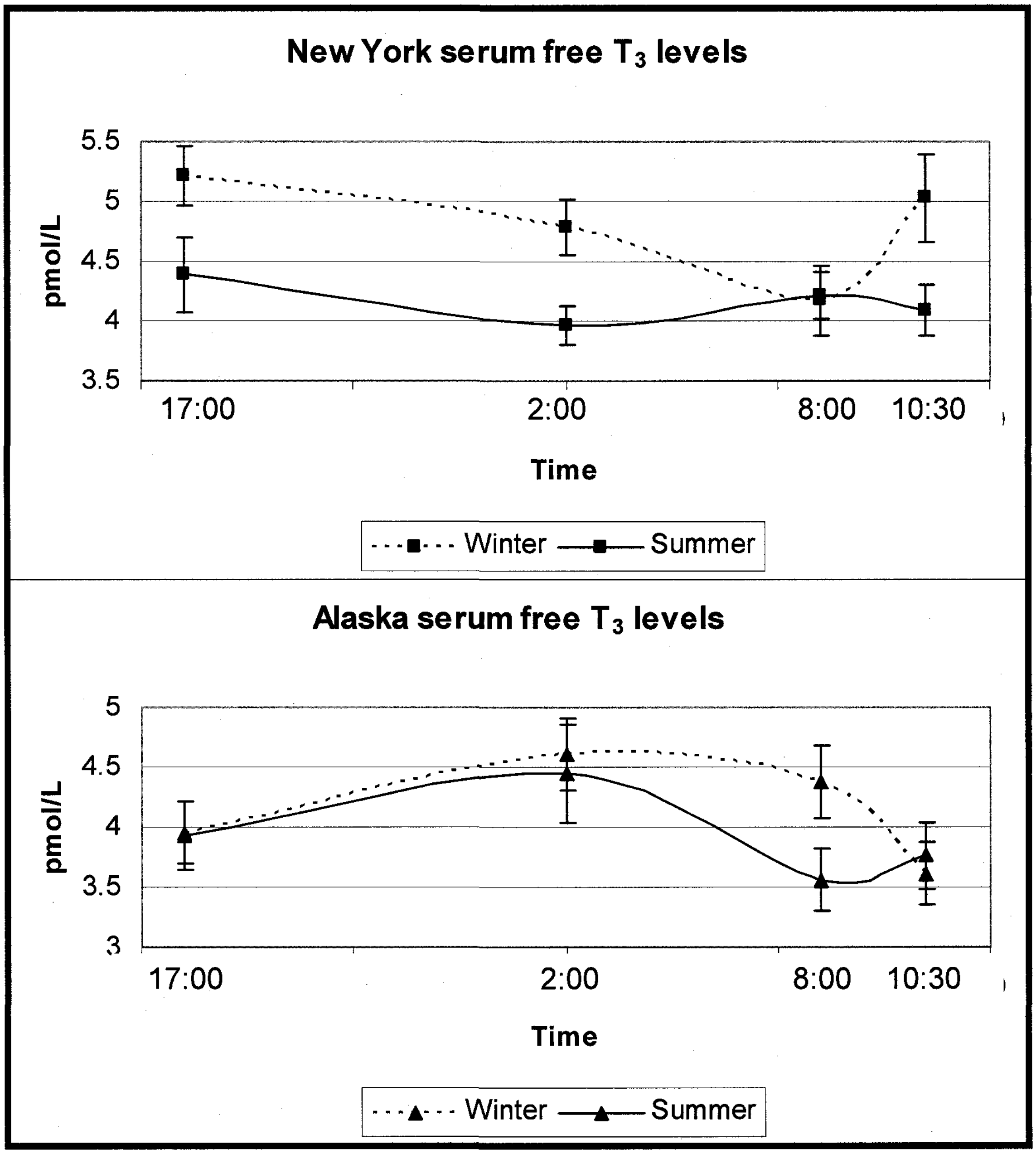

Figure 3.2: Serum free T_3 (TT3) in sled dogs living in New York or Alaska collected on the winter and summer solstice.

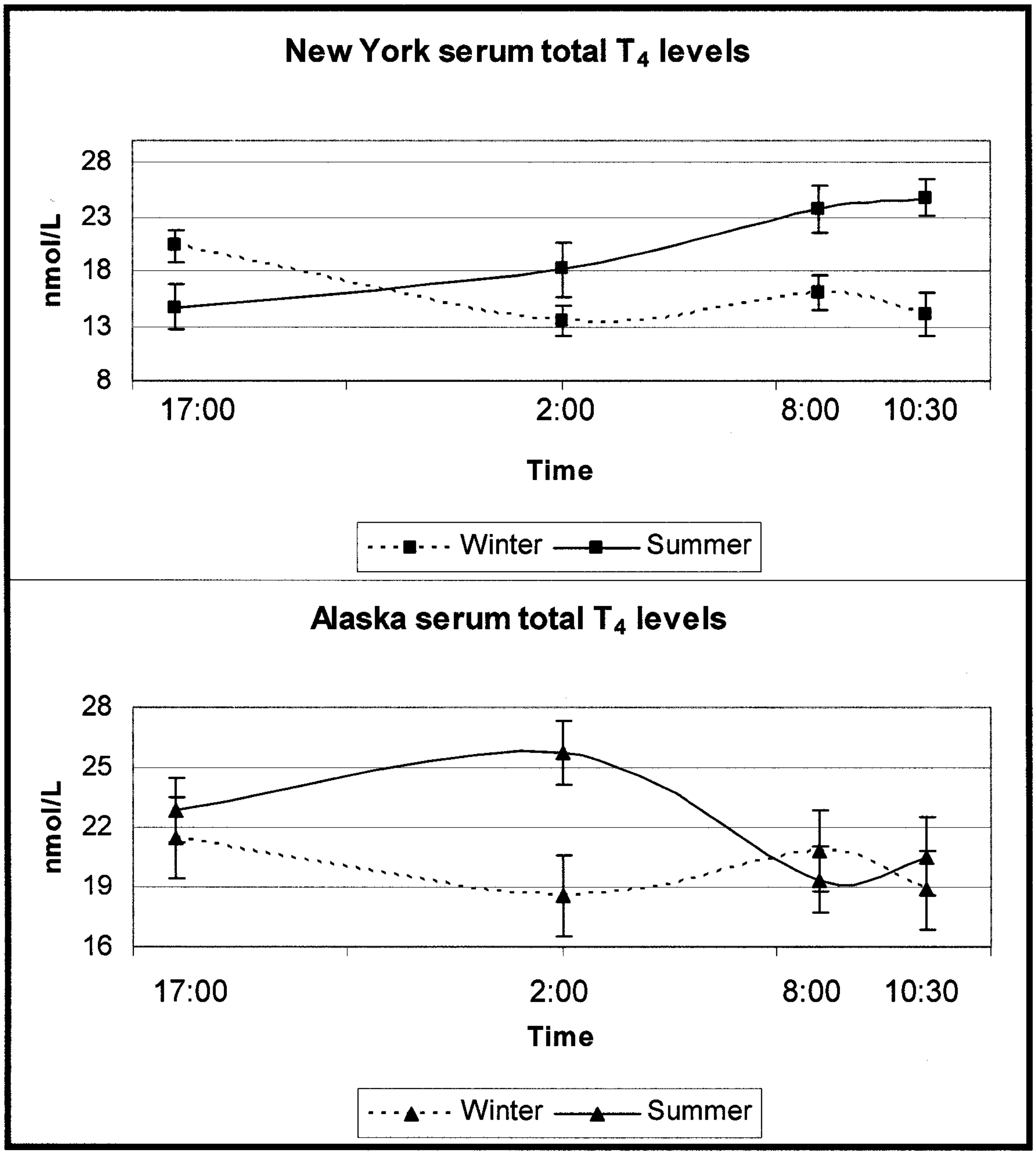

Figure 3.3: Serum Total T_4 (TT4) in sled dogs living in New York or Alaska collected on the winter and summer solstice.

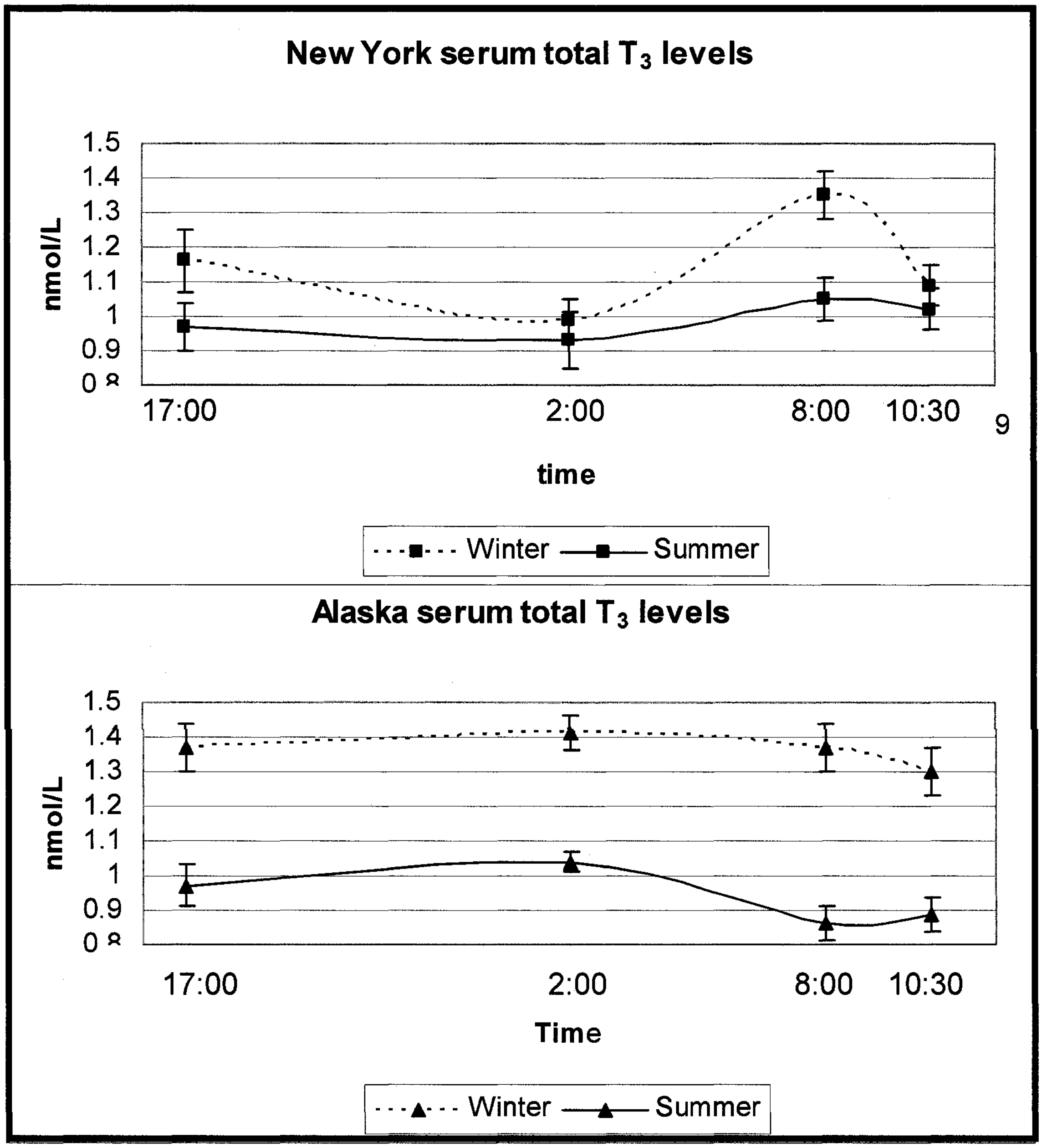

Figure 3.4: Serum total T_3 (TT3) in sled dogs living in New York or Alaska collected on the winter and summer solstice.

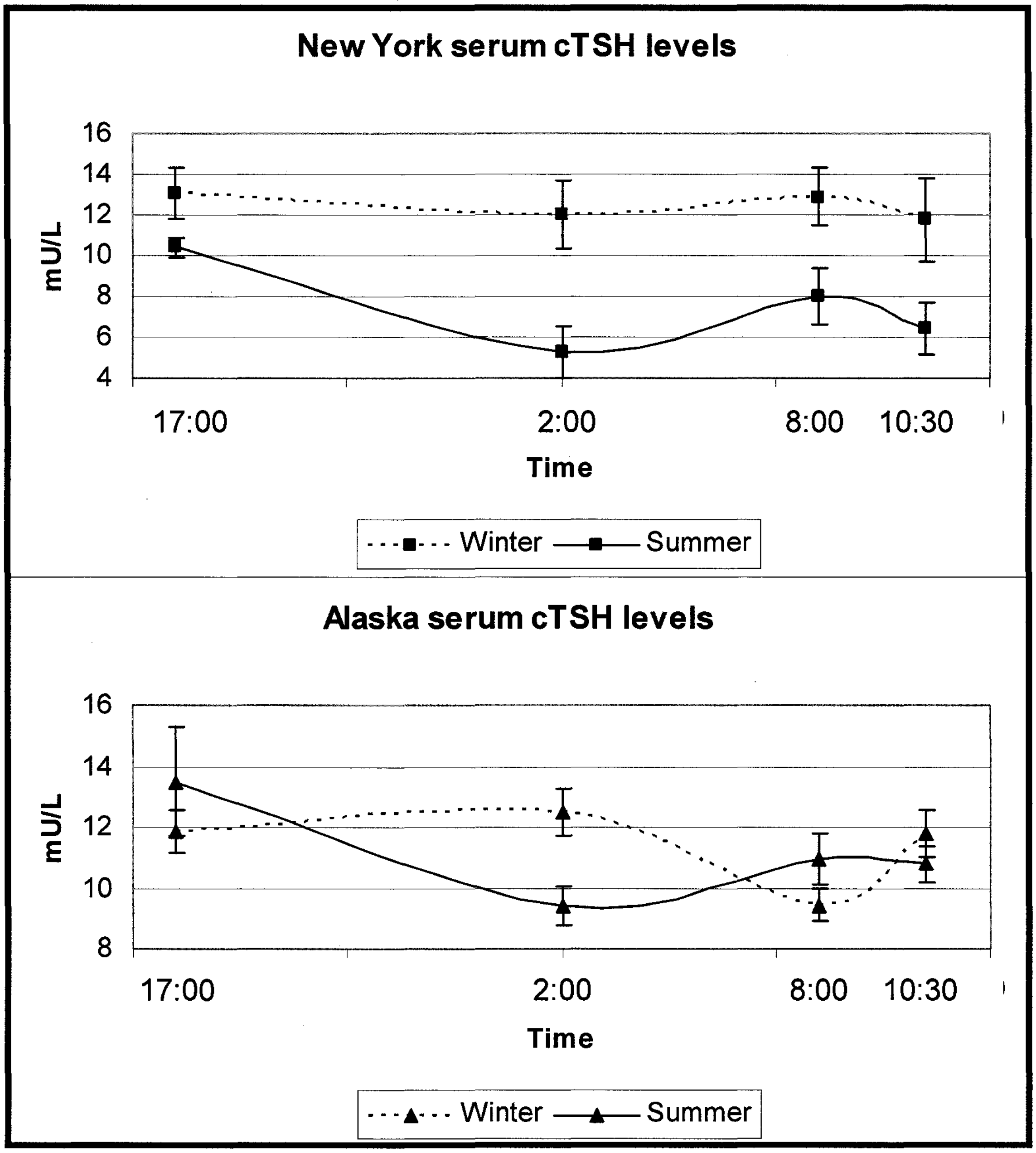

Figure 3.5: Serum thyroid stimulating hormone (cTSH) in sled dogs living in New York or Alaska collected on the winter and summer solstice.

Table 3.1: Mean and SEM of thyroid hormones in sled dogs located in Alaska or New York. Values represent the diurnal variation on the winter and summer solstice. Significance is established with $p \leq 0.05$ and significance is shown with letter superscripts. Capital letter superscripts refer to the values in which the lower case superscripts are compared.

Serum free T_4							
NY winter	mean±sem	NYsummer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
17:00	$12.5\pm1.3^{C,G}$	17:00	$7.71\pm0.9^{a,c,E}$	17:00	12.7 ± 1.0	17:00	11.2 ± 1.1^{A}
2:00	$7.17\pm1.1^{b,g,h}$	2:00	$10.3\pm1.4^{b,e,f}$	2:00	14.0 ± 1.0^{B}	2:00	10.7 ± 0.8
8:00	11.5 ± 1.7^{H}	8:00	13.4 ± 1.4^{e}	8:00	13.3 ± 0.9	8:00	10.7 ± 0.7
10:30	$9.02\pm1.8^{d,g}$	10:30	$15.1\pm1.4^{D,e,F}$	10:30	12.1 ± 1.6	10:30	12.2 ± 1.0
Serum free T_3							
NY winter	mean±sem	NY summer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
17:00	$5.16\pm0.3^{A,D,k}$	17:00	3.93 ± 0.3^{d}	17:00	$3.95\pm0.3^{a,H}$	17:00	3.93 ± 0.3^{e}
2:00	4.73 ± 0.2^{k}	2:00	4.42 ± 0.2	2:00	$4.61\pm0.3^{h,I}$	2:00	$4.43\pm0.4^{E,F,G}$
8:00	$4.18\pm0.3^{K,l}$	8:00	3.56 ± 0.2	8:00	$4.38\pm0.3^{C,h,J}$	8:00	$3.56\pm0.3^{c,f}$
10:30	$5.01\pm0.4^{B,L}$	10:30	3.78 ± 0.2	10:30	$3.61\pm0.3^{b,I,j}$	10:30	3.78 ± 0.3^{g}
Serum total T_4							
NY winter	mean±sem	NY summer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
17:00	19.3 ± 1.4^{H}	17:00	$14.7\pm2.1^{a,F}$	17:00	21.5 ± 2.0^{E}	17:00	22.8 ± 1.6^{A}
2:00	13.9 ± 1.4^{h}	2:00	$18.2\pm2.4^{b,f,G}$	2:00	$18.5\pm2.0^{b,e}$	2:00	$25.7\pm1.6^{B,a}$
8:00	15.9 ± 1.6^{c}	8:00	$23.7\pm2.2^{C,f,g,d}$	8:00	20.8 ± 2.0	8:00	$19.3\pm1.6^{a,b}$
10:30	$14.7\pm1.0^{d,h}$	10:30	$24.8\pm1.7^{D,f,g}$	10:30	18.7 ± 2.0^{e}	10:30	20.5 ± 1.9^{b}
Serum total T_3							
NY winter	mean±sem	NY summer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
17:00	$1.16\pm0.09^{B,I}$	17:00	0.97 ± 0.07	17:00	1.37 ± 0.07^{b}	17:00	0.97 ± 0.06^{e}
2:00	$0.99\pm0.06^{C,i,j}$	2:00	0.93 ± 0.08^{h}	2:00	$1.41\pm0.05^{c,G}$	2:00	$1.04\pm0.03^{e,F}$
8:00	$1.35\pm0.07^{i,J}$	8:00	$1.04\pm0.06^{A,H}$	8:00	1.38 ± 0.07	8:00	$0.86\pm0.05^{a,E}$
10:30	$1.09\pm0.06^{D,j}$	10:30	1.02 ± 0.06	10:30	$1.30\pm0.07^{d,g}$	10:30	0.89 ± 0.05^{f}
Serum thyroid stimulation hormone							
NY winter	mean±sem	NY summer	mean±sem	AK winter	mean±sem	AK summer	mean±sem
17:00	13.8 ± 1.3	17:00	10.4 ± 0.4^{H}	17:00	11.9 ± 0.7^{g}	17:00	13.5 ± 1.8^{F}
2:00	12.6 ± 1.7^{D}	2:00	$5.89\pm1.3^{d,h}$	2:00	$12.5\pm0.8^{C,g}$	2:00	$9.39\pm0.6^{c,f}$
8:00	13.8 ± 1.4^{B}	8:00	7.93 ± 1.4^{b}	8:00	$9.42\pm0.5^{b,G}$	8:00	10.9 ± 0.8^{f}
10:30	12.7 ± 2.1^{c}	10:30	$6.41\pm1.3^{a,E,h}$	10:30	11.8 ± 0.8^{g}	10:30	$10.8\pm0.6^{A,f}$

Chapter 4

Hair analysis in sled dogs (*Canis lupus familiaris*) illustrates a linkage of mercury exposure along the Yukon River with human subsistence food systems*

4.1 Abstract

Because the dog has been an important biomedical research model and hair samples from sled dogs could be used as a biomarker of exposure to metals. In this study, hair samples are used as a non-invasive indicator of mercury exposure in sled dogs fed commercial food and traditional village diets. Sled dog populations living in rural New York and Alaska were collected in 2005 and 2006. Total mercury (THg) content was determined on the entire hair sample in sled dogs from New York and a reference site, Salcha, Alaska. Both sites fed a commercial feed for high performance dogs. Mean THg levels in New York sled dogs were 36.6 ng/g and Alaskan sled dogs, occasionally supplemented with fish oil, showed THg mean of 58.2 ng/g. These THg levels are below levels that are suggested to cause adverse effects and should be considered baseline levels. Yukon river sled dogs had higher THg, ranging from 139 to15,800 ng/g and showed decreasing mean levels from the delta area to upriver. There were significant differences between THg in the dogs from Russian Mission (10908.3±3028 ng/g), the farthest west village, and Ft. Yukon (1822.4±1747 ng/g), the farthest east village.

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*Submitted to Science of the Total Environment, Dunlap KL, Reynolds AJ, Duffy LK, February 2007

4.2 Introduction

Climate change, in combination with socioeconomic change and natural resource development across the circumpolar north has the potential to impact the dietary consumption of human subsistence harvesters. This cumulative stress on ecosystem and cultural services is creating a compelling need for the development of bioindicators for monitoring environmental change (Hoekstra et al., 2003; Sobanska et al., 2005; Dehn et al., 2006). The presence of mercury in northern food chains, especially in traditional foods, has been a concern of health scientists because of its toxicity and bioaccumulation potential (Duffy et al., 2005; Jewett et al., 2003; Braune et al., 1999).

Hair from various mammals has been used as a convenient biomarker of mercury (Hg) pollution, including caribou (Duffy et al, 2005), wild boar (Sobanska, 2005), sea lions (Beckmen et al., 2002), seals (Sun et al., 2006), river otter (Ben-David et al., 2001), opossum (Burger et al., 1994), muskrats (Stevens et al., 1997) and dogs (Hansen and Danscher, 1995). Since mammals share many physiological and biochemical characteristics with humans, Hg could impact the same mechanisms that influence human health. The dog has long been an important research model and is considered an omnivore when associated with humans. Dogs share the social and biophysical environment with humans and develop many of the same diseases, especially immunological syndromes (Dunlap, 2006; Felsburg, 2002), and thus are an ideal animal model for environmental impacts on northern people. In several villages in the North, sled dogs are predominately fed the same type of food as is consumed by humans, including salmon, white fish, caribou, moose, sea mammals, and bear (Andersen, 1992; Hansen and Danscher, 1995).

The objectives of this study are: 1) to investigate the utility of sled dog (*Canis lupus familiaris*) hair as a non-invasive indicator of mercury; and 2) survey the transfer of Hg from fish to a mammal population, more specifically sled dogs, along the Yukon River. This research tested the hypothesis that sled dogs fed commercial dog chow would have lower levels of mercury in their hair then sled dogs fed a more human like diet that included fish.

4.3 Materials and Methods

4.3.1 Hair Samples

Sled dog (*Canis lupus familiaris*) hair was collected in Alaska and New York (2005-2006). About 0.2 grams of front dorsal hair (lower neck) was cut near to the skin (proximal) by means of stainless-steel surgical scissors. Hair samples were washed and homogenized before analysis. The gender and age of the sled dogs were also recorded.

Samples were obtained from several Alaska sites and one New York site: 16 individuals from New York (45˚N) and 20 individuals from Salcha, Alaska (65˚N); both sample populations were fed Purina Pro-Plan, and were sampled in the summer of 2005 as reference kennels. Twelve Yukon River dogs were sampled in the summer of 2006 from each village: Russian Mission (62°N), Galena (64°N), Rampart (65°N), Fort Yukon (66°N), and again from Salcha, Alaska. The Yukon River dogs were maintained on a subsistence diet that consisted primarily of seasonal salmon for at least two months prior to collection. A two month diet-recall with kennel owners was performed at every village. All kennel owners listed salmon as the primary food source and photo documentation of feed barrels and salmon drying racks was performed. Supplemented foods that were given to the dogs at some time in the past 2 months included black bear, pike, moose, chicken and commercial food, in agreement with the more extensive survey of Andersen (1992).

4.3.2 Sample Preparation and QC Method

Hair samples were washed prior to digestion. A benzene wash was performed using 5 consecutive 10 mL rinses of benzene. For the first two rinses, the samples were shaken every 10 minutes for an hour. The final three benzene rinses were performed by shaking the sample for one minute in 10 mL of benzene followed by the removal of the benzene with a pipette. After the washing was complete, the hair was dried overnight in an oven at ~65°C and then homogenized.

Total mercury (THg) concentration in the hair samples were measured at Frontier Geosciences (Seattle, WA) using the cold vapor atomic fluorescence spectrometry (CVAF) method (Duffy et al., 2005). The results are reported on a wet weight (w/wt) basis as ng/g (ppb). All analysis have been run with respect to a thorough quality control program using reference material (DOLT3-dog fish liver tissue) as well as spiked samples. The percent recovery of DOLT3 was 102%. For quality control, two samples were spiked and the percent matrix spike recovery average was 84% (82% and 86%).

4.3.3 Sample Digestion

For total mercury in tissue, approximately 0.1-0.2 g of each dog hair sample was digested with 10 mL of hot refluxing 70% HNO_3: 30% H_2SO_4 for approximately two hours. The digests were then diluted to a final volume of 40 mL with a solution of 10% (v/v) 0.2 N BrCl. Following complete digestion on a hotplate, the samples were diluted up to 40 mL with methanol (neat). From each KOH methanol digest, an aliquot of 0.5 mL was removed and aliquoted into a 60 mL Teflon distilling vial containing 45 mL of DDI water.

4.3.4 Total Hg Analysis

Aliquots of each digest were reduced in pre-purged double-distilled water to Hg° with $SnCl_2$, and then the Hg° purged onto gold traps as a preconcentration step. The Hg contained on the gold traps was then analyzed by thermal desorption into a cold vapor atomic fluorescence detector (CVAF) using the dual amalgamation technique.

4.3.5 Statistical Analysis

One way ANOVA was performed to compare the THg between the New York group and the Alaska group. Significance was established with a P value less than 0.05. In the Alaska 2005 reference group, two outlying data points were removed from the analysis because they were more than four times the mean (Gamberg and Braune, 1999). Samples from the Alaskan 2006 reference site and the Yukon River villages were analyzed using SAS statistical software. Analysis of variance was used to analyze all the data to evaluate the effects of sampling location on THg concentrations in hair. One sample was removed from analysis from the Salcha group because the sample was more than four times the mean (Gamberg and Braune, 1999). Significant differences between sampling sites was determined using Tukey's Studentized range test. Differences were considered significant at $P \leq 0.05$.

4.4 Results

The THg levels of the sled dogs fed commercial diets in 2005 are reported in Table 4.1. The mean concentration of 36.6 ng/g ±13.6 for the New York sled dogs was lower than the THg mean concentration for the Salcha, Alaska sled dogs which was 58.2 ng/g. The range for the Salcha sled dogs was 45.2-80.4 ng/g and the range for the New York sled dogs was 20.0-59.5 ng/g. Comparisons of these two commercial fed groups show that the Salcha group had more females (40% vs. 25%). While the Salcha group had a wider range of ages, both groups had similar mean ages (3.6±2.7 years vs. 3.9±3.9 years). There was no correlation of age with THg concentration.

The THg levels in Yukon River sled dogs (Table 4.2) progressively decreased as samples were collected upriver from the river delta (Fig 4.1). The reference dogs in Salcha had significantly lower THg levels compared to all sampling sites, with the exception of Ft. Yukon (Table 4.3, Fig 4.1). Russian Mission sled dogs had significantly higher THg concentration than all other sampling sites. For intermediate sites, significant differences were found between Galena and Ft. Yukon (Table 4.3).

4.5 Discussion

Average mercury concentrations shown in Table 4.1 for these sled dogs falls within the range for domestic dogs (Eisler, 2006). No data points from any dog approached concentrations indicative of potential toxic effects (30,000 ng/g; Thompson, 1996). Smith and Armstrong (1975) reported that Innuit sled dogs, subsisting largely on seal meat, contained levels of Hg in liver, up to 11,500 ng/g, without apparent harm.

Smaller mammals such as mink, cats, dogs, and river otters appear to be more resistant to Hg than are larger mammals (Eisler, 2006). Eisler (2006) suggests that these differences are related to metabolism and possible Hg detoxification rates. For mammals, the main concern is about Hg exposure levels during the early stages of pregnancy— the first trimester in humans. Khera (1979) reported that domestic dogs exposed to 0.1 mg/kg to 0.25 mg/kg of body weight during their entire pregnancy showed a higher incidence of stillbirths. The mean values of THg for the Yukon River villages exceed the no effects hazard concentrations for river otters (660 ng/g) and both mean levels for Russian Mission and Galena exceed the low effects hazard concentration (3290 ng/g) (Hinck et al., 2006). Since sled dogs are usually larger than river otters, the current risk to the sled dogs through the consumption of fish is minimal although biomarkers of cardiovascular and immune functions remain to be examined.

Published data on mercury in dog hair (or *Canis lupus*, in general) is sparse (Hoekstra et al., 2003; Gamberg and Braune, 1999; Hansen and Dansher, 1995). In a study on sled dogs, Hansen and Dansher (1995) reported mercury levels in the hair of 10 dogs fed meat from marine mammals. The hair concentration ranged from 4,105 to

34,743 ng/g, which is twice as high as our observed values in Russian Mission (Table 4.2). Hair THg levels were also higher than concentrations in liver and kidney in their younger dogs. Based on Hansen and Dansher (1995) the tissue distribution of THg levels in dogs exposed to high levels of THg are: hair: muscles (1:0.05); hair: liver (1:0.13); hair: kidney (1:0.25). Our results support Hansen's study in that we observed significant difference between the fish fed dogs and commercially fed dogs from both New York and Salcha, Alaska (ANOVA, $p<.001$). The hair THg concentration in the village dogs ranged from 139 to15,800 ng/g.

Hansen and Danscher (1995) found a biphasic relationship between THg hair concentration and age. We found no relationship with age. Hg enters the food chain in the north as input from both regional geology and atmospheric transport (Nriagu, 1989; Peterson et al., 2007). Hg is generally found at greater concentrations in higher trophic level marine biota relative to terrestrial mammals due to the biomagnification of organic mercury forms and a longer food chain (Eisler, 2006). While interspecies comparisons of THg are difficult because of feeding selection and behavior, our data may serve as reference for closely related terrestrial species such as wolves, fox and wolverines in Alaska.

Sled dog hair from Russian Mission, about 260 km from Norton Sound on the Bering Sea, had the highest mean level of THg in their hair (10,908 ng/g) and Fort Yukon, some 1300 km up river from the delta, had the lowest (1,822 ng/g) THg concentrations (Table 4.2). Galena (4,528 ng/g) and Rampart (2,446 ng/g) were intermediate. In particular, the THg levels in Russian Mission were among the highest reported from hair in Alaska, similar to those reported in hair for Stellar sea lions (Beckmen et al., 2002), fur seals (Beckmen et al., 2002), and river otters (Ben-David et al., 2001). Note that the concentrations and patterns in Salcha, the reference site, are 10-fold lower than the sled dogs with the coastal subsistence diet. Mercury concentrations in fish can be highly variable in subarctic waters (Zhang et al., 2001; Jewett et al., 2003) and can differ with ecosystem structure (Ben-David, et al. 2001), wetland areas (Hinck et al., 2006) and forest fire frequency (Taylor, 2007). The presence of large salmon runs is related to the diet of the dogs (and humans) along the Yukon and needs to be monitored as system dynamics change; there are several important drivers of this process, including the marine-nutrient pump (Krummel et al., 2003).

The amount of THg transported by fish to sled dogs appears greater at the river delta. The spawning salmon act as contaminant pumps by transporting Hg inland (Zhang et al., 2001). Sampling was done August through September when salmon are plentiful and abundant in diets fed to the dogs. Hair samples in sled dogs however, are representative of the past year of mercury accumulation, when dogs are fed dried fish, caribou and other supplements. Decreasing THg concentrations upstream from the river delta may be due to salmon availability for the rest of the year as well as other supplemented subsistence foods. All participating mushers fed salmon when available and supplemented the diet when supply was limited and when race season began. The number of salmon, both available and harvested, is greater in number and variation the closer you are in proximity to the mouth of the Bering Sea. A second possibility for the decreasing trend as you travel up stream from the delta may be a consequence of contaminant elimination. During migration, salmon stop eating and rely on protein and lipids stores. As these stores are mobilized for energy use, Hg bound to protein and lipids may also be mobilized and eliminated from the animal. More research is needed to understand the flow of THg from fish to ecoreceptors, including subsistence users.

As climate change impacts global marine systems and wildfire regimes, the release of Hg and other toxic trace elements may increase (Patz, 2005). Continued monitoring of contaminant concentrations is needed to document trends in exposure to subsistence users. This type of study was initiated years ago, when Elders from the Yukon-Kuskokwim drainages wondered if metals such as Hg posed a health threat. They knew Hg could accumulate in fish and be transferred up the food chain. They also knew that Hg has always been present in the environment (Gerlach et al., 2006) but wondered how the increased development such as mining and subsequent erosion of geological deposits would affect their food, and thus the health of future generations. Unfortunately we still cannot fully answer that question.

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4.7 References

Andersen DB. The use of dog teams and the use of subsistence-caught fish for feeding sled dogs in the Yukon River drainage, Alaska. Alaska Department of Fish and Game Technical Paper No. 210; 1992.

Beckmen KB, Duffy LK, Zhang X, Pitcher KW. Mercury concentrations in the fur of Stellar sea lions and northern fur seals from Alaska. Mar Pollut Bull 2002; 44: 1130-5.

Ben-David M, Duffy LK, Blundell GM, Bowyer RT. Natural exposure of coastal river otters to mercury: relation to age, diet, and survival. Environ Toxicol Chem 2001; 20(9): 1986-92.

Braune B, Muir D, Demarch B, Gamberg M, Poole K, Currie R. Spatial and temporal trends of contaminants in Canadian Arctic freshwater and terrestrial ecosystems: a review. Sci Total Environ 1999; 230: 145-208.

Burger J, Marquez M, Goechfeld M. Heavy metals in the hair of opossum from Palo Verde, Costa Rica. Arch Environ Contam Toxicol 1994; 27(2): 154-61.

Dehn LA, Follmann EH, Thomas DL, Sheffield GG, Rosa C, Duffy LK, O'Hara TM. Trophic relationships in an Arctic food web and implications for trace metal transfer. Sci Total Environ 2006; 362(1-3): 103-23.

Duffy LK, Duffy RS, Finstad G, Gerlach G. A note on mercury levels in the hair of Alaskan reindeer. Sci Total Environ 2005; 339: 273-6.

Dunlap KL, Reynolds AJ, Duffy LK. Total antioxidant power in sled dogs supplemented with blueberries and the comparison of blood parameters associated with exercise. Comp Biochem Physiol A Mol Integr Physiol 2006; 143(4): 429-34.

Eisler R. Mercury hazards to living organisms. Taylor and Francis Group, Boca Raton, FL, 2006, 312 pp.

Felsburg PJ. Overview of immune system development in the dog: comparison with humans. Human Exper Toxicol 2002; 21: 487-92.

Gamberg M, Braune BM. Contaminant residue levels in arctic wolves (*Canis Lupus*) from the Yukon Territory, Canada. Sci Tot Environ 1999; 243/244: 329-38.

Gerlach CS, Duffy LK, Murray MS, Bowers PM, Adams R, Verbrugge DA. An exploratory study of total mercury levels in archaeological caribou hair from northwest Alaska. Chemosphere 2006; 65: 1909-14.

Hansen JC, Danscher G. Quantitative and qualitative distribution of mercury in organs from arctic sledgedogs: an atomic absorption spectrophotometric and histochemical study of tissue samples from natural long-term high dietary organic mercury-exposed dogs from Thule, Greenland. Pharmacol Toxicol 1995; 77: 189-95.

Hinck JE, Schmitt CJ, Echols KR, May TW, Orazio CE, Tillit DE. Environmental contaminants in fish and their associated risk to piscivorous wildlife in the Yukon River Basin, Alaska. Arch Environ Contam Toxicol 2006; 51(4): 661-72.

Hoekstra PF, Braune BM, Elkin B, Armstrong FJ, Muir DG. Concentrations of selected essential and non-essential elements in Arctic fox (*Alopex lagopus*) and wolverines (*Gulo gulo*) from the Canadian Arctic. Sci Tot Environ 2003; 309: 81-92.

Jewett SC, Zhang X, Naidu AS, Kelley JJ, Dasher D, Duffy LK. Comparison of mercury and methylmercury in northern pike and Arctic grayling from western Alaska rivers. Chemosphere 2003; 50(3): 383-92.

Khera KS. Teratogencity and genetic effects of mercury toxicity. In: Nriagu JO, editor. The biogeochemistry of mercury in the environment. Elsevier/North Holland Biomedical Press, New York, NY, 1979: 501-18

Krummel EM, Macdonald RW, Kimpe LE, Gregory-Eaves I, Demers MJ, Smol JP, Finney B, Blais JM. Aquatic ecology: delivery of pollutants by spawning salmon. Nature 2003; 425: 255-6.

Nriagu JO. A global assessment of natural sources of atmospheric trace metals. Nature 1989; 338: 47-9.

Patz JA. Climate change. In: Frumkin H, editor. Environmental health: from global to local. Jossey-Bass, San Francisco, CA, 2005: 238-68.

Peterson SA, Sickle J, Herlihy AT, Hughes RM. Mercury concentration in fish from streams and rivers throughout the western United States. Environ Sci Technol 2007; 41: 58-65.

Smith TJ, Armstrong FAJ. Mercury in seals, terrestrial carnivores and principal food items of the Inuit, from Holman N.W.T. J Fish Res Bd Canada 1975; 32: 795-801.

Sobanska MA. Wild boar hair (*Sirs serofa*) as a non-invasive indicator of mercury pollution. Sci Tot Environ 2005; 339: 81-8.

Sun L, Yin X, Liu X, Zhu R, Xie Z, Wang Y. A 2000 year record of mercury and ancient civilizations in seal hairs from King George Island west Antarctica. Sci Total Environ 2006; 368: 236-47.

Stevens RT, Ashwood TL, Sleeman JM. Mercury in hair of muskrats and mink from US Department of Energy Oak Ridge Reservation. Bull Environ Contam Toxicol 1997; 58: 720-5.

Taylor DA. Forest fire fallout. Environ Health Perspect 2007; 115(1): A21

Thompson DR. Mercury in buds and terrestrial mammals. In: Beyer WN, Heing GH, Redmon-Norword AW, editors. Environmental Contaminants in Wildlife: Interpreting tissue concentrations. CRC Press, Boca Raton, Florida, 1996: 341-56.

Zhang Y, Naidu AS, Kelley JJ, Jewett SC, Dasher D, Duffy LK. Baseline concentrations of total mercury and methylmercury in salmon returning via the Bering Sea (1999-2000). Mar Pollut Bull 2001; 42(10): 993-7.

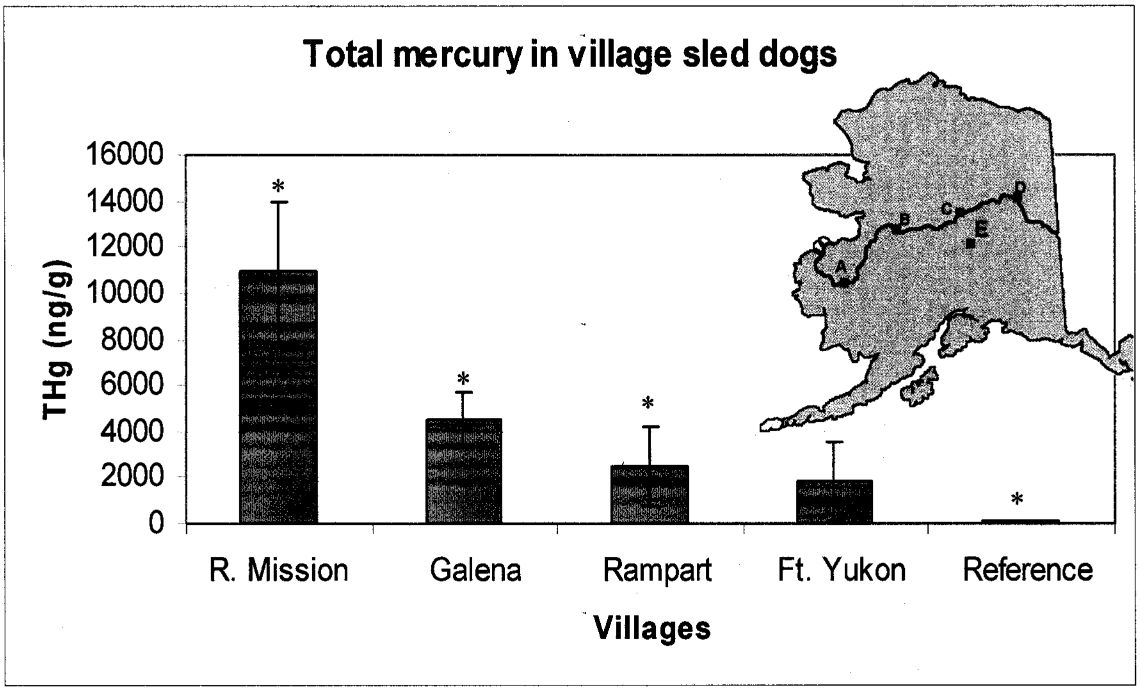

Figure 4.1: Total mercury concentrations in hair in village sled dogs subsisting on local salmon along the Yukon River and mercury concentrations from a reference kennel in Salcha, Alaska. Asterisks (*) indicate villages that have significantly greater THg than the reference kennel. On the inset map, (A) is Russian Mission, (B) is Galena, (C) is Rampart, (D) is Fort Yukon and (E) is Salcha, where the reference kennel is located. Comparisons made between all sampling locations are shown in Table 3.

Table 4.1: Total mercury concentrations (ng/g) in hair samples in New York and Alaskan sled dogs maintained on commercial feed (Purina Pro Plan Performance®).

Dog	Alaska	New York
1	51.4	20.2
2	53.9	22.4
3	57.8	45.0
4	63.8	20.0
5	60.3	20.3
6	65.5	28.1
7	58.9	35.6
8	65.4	59.3
9	68.6	23.0
10	60.7	46.5
11	55.0	38.5
12	53.4	52.2
13	45.2	36.1
14	44.2	40.6
15	66.3	59.5
16	45.2	38.5
17	52.0	
18	80.4	
Mean*	**58.2±9.3**	**36.6±13.5**

*Mean THg levels in Alaska were significantly higher than in New York ($p = 0.000005$)

Table 4.2: Total mercury concentrations (ng/g) in hair samples in subsistence fed sled dogs in 4 different villages along the Yukon River (Russian Mission, Galena, Rampart, Fort Yukon) and a reference site (Salcha), maintained on a commercial feed (Purina Pro Plan Performance®).

Dog	R. Mission	Galena	Rampart	Ft. Yukon	Salcha
1	12200	4100	6010	2510	125
2	12100	3740	3250	269	63.1
3	13700	4630	1680	3780	85.6
4	7830	5310	1000	139	79.9
5	8880	5450	2190	2720	78.4
6	12500	5330	4260	3980	NU
7	15800	4350	491	145	78.9
8	12900	3930	4720	1800	102
9	10600	6410	1350	587	101
10	4420	ND	1170	521	115
11	11000	2120	2170	5050	75.7
12	8970	4440	1070	368	97.1
Mean	**10908.3±3028**	**4528.2±1125**	**2446.8±1737**	**1822.4±1747**	**91.1±18.6**

ND sample from Galena was not detected and NU in Salcha was omitted because it was more than 4 times the mean.

Table 4.3: Matrix table showing significant relationships between village dogs along the Yukon River (Russian Mission, Galena, Rampart, Fort Yukon) and the reference kennel in Salcha.

	R. Mission	**Galena**	**Rampart**	**Ft. Yukon**	**Salcha**
R. Mission	---	S	S	S	S
Galena	S	---	NS	S	S
Rampart	S	NS	---	NS	S
Ft. Yukon	S	S	NS	---	NS
Salcha	S	S	S	NS	---

Significant interactions are indicated with an (S), while non-significant interactions are indicated with an (NS).

Chapter 5

Yukon River sled dogs illustrate a linkage between ecosystem, mercury, and human subsistence systems*

5.1 Abstract

Before western diets, circumpolar people had a low incidence of obesity, diabetes, and cardiovascular disease. In contrast to risks associated with a high fat, high protein diet, health benefits are attributable to a subsistence diet that is rich in omega-3 fatty acids and antioxidants. While subsistence diets have been shown to provide substantial health benefits, there are also risks associated with them as a result of industrialization and the widespread distribution of chemicals in the environment. Native people and their sled dogs are exposed to a variety of contaminants that accumulate in the fish and game that they consume. The sled dogs in these villages are maintained on the same subsistence foods, primarily salmon, and therefore they can be used as models for researching the effects that a subsistence diet might have on immune parameters. Several biomarkers of health status— acute phase proteins, antioxidant status, and eicosanoid production— were measured in village sled dogs along the Yukon

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River as markers of immune function and inflammation. A reference kennel, maintained on a nutritionally balanced commercial diet, was also measured for comparison. The health indicators such as antioxidant status were inversely correlated with mercury exposure.

5.2 Introduction

Rural Alaska communities typically live a subsistence lifestyle to survive and to uphold traditional, cultural, and spiritual values. Pollution from other regions of this modern world has found its way to these northern communities by wind, water or wildlife and many believe that these pollutants have tainted their food sources (1-3). Despite these contaminants, traditional foods such as rich colored berries, native plants, wild game and salmon, compounded with the physical endurance needed to live such a lifestyle, provide many health benefits (2,3). But to what extend do these nutrient rich foods and physical endurance combat the hidden 'risks' associated with environmental toxins in their food sources?

Sled dog mushing, once used only as a means of transportation, has evolved into a modern sport. Though Alaska is known for sled dog racing, this sport spans the country and across continents. In fact, the earliest organized sled dog race was held in Laconia New Hampshire, and today there are sled dog clubs in remote locations such as Africa and Australia. Such diversity of climate and diet provide an ideal opportunity to undertake nutrition, exercise, physiology and toxicology research. Sled dogs are unique research models because they can be found all over the world in relatively large numbers of genetically similar animals, that are exercised and housed in similar conditions. Because of this, diet, the effects of exercise, disease, and environment, can be observed on the cardiovascular and immune systems. Dogs, in general, have become a popular model for aging, immune function, toxicology and cognitive disorders (4-8). Dogs have shown to have key features associated with cognitive dysfunctions, beta-amyloid pathology, and oxidative damage similar to that of humans (7).

Sled dogs in northern climates are often exposed to the same environmental hazards as are their human counterparts (9,10). In many Alaskan villages sled dogs are still a fundamental part of a traditional lifestyle, used for trapping, packing and transportation, although far less used than snow machines for winter travel today. Most of these villages are small settlements, established on or near rivers to facilitate travel and to gather their food supply. The diet of both the natives and their sled dogs in Alaska is often comprised of a variety of wild game, fish and marine mammals. In addition to being a fundamental component of the native diet, salmon is the primary food source for sled dogs throughout the year (11).

Before western diets, circumpolar people had a low incidence of obesity, diabetes, cancer and cardiovascular disease (2,12-15). Contrary to the risks associated with a high fat, high protein diet, health benefits can also be attributed to a diet rich in omega-3 fatty acids and antioxidants, offered from indigenous foods (2,16-18). Diets high in antioxidant-rich foods are associated with reduced risk of cardiovascular diseases, cancers, and Alzheimer's disease (19,20). A group of Greenlandic Eskimos that existed on a diet made up primarily of fish and sea mammals, high in omega-3 fatty acids displayed a remarkably low prevalence of cardiovascular disease (13). The researchers attributed these findings to the active antithrombotic activity of omega-3 fatty acids (12,13).

Fish and sea mammals are a rich source of omega-3 fatty acids, namely eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (17,21). Several studies have shown that diets high in EPA and DHA display a marked increase in the incorporation of EPA and DHA into the membranes of cells involved in inflammation (21,22). Arachidonic acid (AA; 20:4n-6) in the cell wall is cleaved by phopholipase A_2 to form eicosanoids, which are modulators of inflammation and immunity. Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), lipoxins, hydroperoxy-eicosatetraenoic acids and hydroxyeicosatetraenoic acids (HETEs). PG and TX are produced by cyclooxygenases enzymes (COX 1 and COX 2) and LT related compounds are produced by lipoxygenase enzymes (LOX) (22,23). EPA and DHA compete with AA for incorporation into cell membranes (23). Omega-3 and omega-6 fatty acids share the same enzymes for the synthesis of LTs and PGs (24), but EPA and DHA act as antagonists to AA by forming less active eicosanoid products.

As substrates for eicosanoid synthesis, EPA and DHA are metabolized to lipid peroxides (21). Omega-3 fatty acids have been shown to increase the production of malonaldehyde, a marker of oxidative damage (25), and therefore, it may be expected that omega-3 fatty acids would have a pro-inflammatory effect (21). In addition, dietary fish oil leads to a decrease in PG synthesis (25). Studies have shown that supplementation with omega-3 fatty acids or fish oil has an immunosuppressant effect.

Though results are varied, most studies have shown that diets high in omega-3 fatty acids cause a decrease in markers of inflammation (21,25,26). The mechanism for the immunosuppressant effect of omega-3 fatty acids is unclear and appears more complex than simply acting as a PG antagonist.

PGs are not the sole mediators produced from AA. LTs are also a product of AA metabolism and if omega-3 fatty acids act as antagonist to the production of specific LTs, then it would be expected that there would be decreases in inflammatory markers (22). The metabolites of EPA are less inflammatory and chemotactic than that of AA. Omega-3 supplementation has shown to decreases LTB_4 production in humans (24), hamsters (27) and rats (28).

In order to quantify potential and/or observed deleterious health affects in sled dogs maintained on a subsistence diet, it is necessary to have reliable biomarkers for immune and cardiovascular function. In addition to LTB_4, C-reactive protein (CRP), an acute phase protein stimulated by the pro-inflammatory cytokine, Il-6 (29), and antioxidant status are useful biomarkers to survey health status (19,20). Since there is an increasing concern with the possible health implications surrounding subsistence food and contaminants in the ecosystem, the need to assess the effects of a subsistence diet on the user's health in relationship to global contaminants such as mercury has been stressed by elders (3,30). The Yukon River system is ideal for such a naturalistic survey because it traverse the state and provides a diversity of native cultures (Fig. 1).

5.3 Results

Sled dogs living in Galena, Alaska had significantly higher LTB_4 levels than all other villages, including the reference kennel (Table 5.1). LTB_4 levels between all other villages did not differ (Fig. 5.2, Table 5.1). Sled dogs living near Rampart, Alaska had the highest mean C-reactive protein (CRP) concentrations that were significantly higher than Galena, Fort Yukon, and Salcha, but not significantly higher than Russian Mission (Fig. 5.2, Table 5.1).

Total Antioxidant Power (TAP) in village sled dogs did not differ significantly between locations. However, sled dogs from Salcha, maintained on commercial food, had significantly higher TAP then all villages (Fig. 5.2, Table 5.1).

The THg levels in Yukon River sled dogs progressively decreased as samples were collected upriver from the river delta. The reference dogs in Salcha had significantly lower THg levels compared to all sampling sites, with the exception of Fort Yukon. Russian Mission sled dogs had significantly higher THg concentration than all other sampling sites. For intermediate sites, significant differences were found between Galena and Ft. Yukon (Fig. 5.2, Table 5.1). In addition, plasma TAP negatively correlated ($P = 0.026$) with THg concentrations in the hair of sled dogs (Fig. 5.2).

5.4 Discussion

Published data on mercury in dog hair (or *Canis lupus*, in general) is sparse (9,31,32). In a study on sled dogs, Hansen and Danscher (9) reported mercury levels in the hair of 10 dogs fed meat from marine mammals. The hair concentration ranged from

4.105×10^3 to 34.743×10^3 ng/g, which is twice as high as our observed values in our Russian Mission samples. The hair THg concentration in the village dogs ranged from 0.139×10^3 to15.800×10^3 ng/g (10).

The current risk at these levels to both humans and their sled dogs through the consumption of fish is not clearly understood, but biomarkers of immune status and inflammation may reveal potential hazards of mercury exposure. Some potential risks associated with human health include cardiovascular disease, impaired immune function, and decreased neural and motor development in children (1,3). We measured three parameters associated with immune function and inflammation in village sled dogs that were exposed to mercury through subsistence food: LTB_4, CRP and TAP.

Neither the leukotriene, LTB_4, nor the acute phase protein, CRP correlate with mercury exposure in the current study. Elevated levels of both these parameters are associated with inflammation. Diets high in omega-3 fatty acids, as is with subsistence diets, have shown a reduction in inflammatory markers (2,24). Most village sled dogs did not have significantly higher inflammatory markers from the reference kennel, suggesting that compounds in subsistence foods are as beneficial as the scientifically derived diet with added antioxidants. However, Galena sled dogs had significantly higher LTB_4 than all villages except Rampart, including the reference kennel. The 2 fold increase in plasma LTB_4 may indicate that these dogs were stressed by local conditions compared to other village dogs.

Likewise, CRP was significantly higher in Rampart than all villages except Russian Mission, including the reference kennel. The most stressed sled dogs, as determined by inflammatory markers, were located in the intermediate sites between the river delta and farthest east village of Fort Yukon. This could be a result of individual animal husbandry, but may also be due to food composition. Unlike the mercury analysis, the blood parameters measured are indicative of the animal's current health status.

Migrating salmon are in a starved state and must use their body reserves to arrive at their destination. Though salmon closest to the river delta may have the highest THg concentrations, they are also relatively strong and healthy, just beginning their travels. Additionally, their nutrient composition, including their polyunsaturated fatty acid profile may be at an optimum. As they migrate their lipid reserves are mobilized, and the beneficial effects of polyunsaturated fatty acids against the toxic affects of mercury may be dampened. Even though THg concentrations in Fort Yukon were 20 times higher than the reference kennel, there may not be deleterious health effects from contaminant exposure and additional analysis of food samples, lipid profiles and additional immune parameters would be useful in understanding the flow of THg from fish to ecoreceptors, including subsistence users.

We observed a highly correlated inverse relationship between mercury exposure and antioxidant status ($P = 0.026$). The production of free radicals and peroxidized lipids by Hg is thought to be a potential mechanism in the formation of cardiovascular disease. Mercury is capable of inducing lipid peroxidation through oxidation of sulfhydryl groups in enzymes, ion channels and receptors, interfering with antioxidants systems, such as glutathione (1,2). The inverse relationship observed in sled dogs, supports this phenomenon. As mercury concentrations decreases, TAP increases, suggestion less interference with endogenous antioxidant defense systems.

It is extremely difficult to establish a cause and effect relationship between contaminant exposure and overall health because of the myriad of other influences and cumulative effects. Regardless, indigenous populations are keenly aware of changes in their environment and food supply (3,29,33). Much of the contamination in subsistence foods is derived from pollutants that are not used in Alaska, indicating that this is a global concern. Contaminant circulation is further complicated with the large market for Alaskan wild salmon. Though the effects of contaminants have an extensive web, the Native population is at greater risk due to their high reliance on indigenous foods. Continued monitoring of contaminant exposure and health effects associated with subsistence diets is necessary.

5.5 Material and Methods

5.5.1 Animals

Alaskan huskies, *Canis lupis familiaris* raised in 4 villages along the Yukon River in Alaska and a reference kennel located in Salsha, Alaska (Latitude, 65°N) were used as test subjects (Fig. 1). The Institutional Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#04-16). There were 12 dogs sampled at each site, including the reference kennel. The village dogs were in or around Russian Mission (62°N), Galena (64°N), Rampart (65°N), and Fort Yukon (66°N). The dogs that were used in this study were typical racing sled dogs with similar lineage and age range.

Housing arrangements varied from kennel to kennel, but most of the dogs were tethered with 2-m chains with access to shelter, water and food.

5.5.2 Diet

Village dogs were primarily maintained on subsistence diets, which included black bear, moose, pike, and salmon. Some diets were supplemented with donated human food from the community and commercial feed within the past 2 months. However, easily 90% of the food source in all villages in the past 2 months was seasonal cooked salmon from local salmon runs. The amount of food fed to each dog was determined individually by the kennel owner but most dogs were maintained at an ideal body condition. Ideal body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum (34, 35). On the sampling day dogs were fed at least 12 hours prior to blood collection to insure that the dogs were in a post-absorptive state.

5.5.3 Blood Sampling

All dogs were bled between 11:30 and 16:00, between August, 26th and October, 7th 2006. Blood was drawn by venipuncture from the cephalic into a 12ml syringe. The blood was then separated into five 3 ml vacutainer tubes. Blood samples were centrifuged at 2500 X g for 10 min on site, transferred into freezer vials, flash frozen in liquid nitrogen and stored at –70°C until they were analyzed.

5.5.4 Biochemical Analysis

The biochemical analyses, including leukotriene B_4, C-reactive protein and total anti-oxidant power, were performed at the University of Alaska Fairbanks.

A commercially available competitive enzyme immunoassay from Cayman Chemical was used to determine plasma leukotriene levels (Catalog # 520111). Samples were collected in vacutainers containing EDTA and indomethacin to a final concentration of 10μM to prevent *ex vivo* formation of eicosanoids. The principal of this assay employs a LTB_4 tracer conjugated to acetylcholinesterase that competes with LTB_4 in the sample to be bound to an antibody specific to LTB_4. A reagent is then added that forms a complex with acetylcholinesterase to form a bright yellow color. The color is inversely proportional to the amount of LTB_4 in the sample. The procedure provided with the assay was followed and a serial dilution was performed prior to running the samples to determine the correct sample amount to be used and to ensure that there was no cross-reactivity.

C-reactive protein was determined in serum samples using a commercially available ELISA kit from BD Biosciences (catalog #557826). This kit is a solid phase sandwich ELISA that utilizes an antibody specific for canine CRP pre-coated on the well plates. After an appropriate incubation period, horseradish peroxidase conjugated to anti-canine CRP is added to produce antibody-antigen-antibody “sandwich”. The addition of a substrate produced a blue color in proportion to the amount of CRP present in the sample. The procedure supplied with the ELISA kit was followed.

A commercial assay from Oxford Biomedical Research laboratory (#TA 01) was used to determine plasma total antioxidant power, using plasma samples collected in vacutainers containing Na^+ citrate. In this assay, the ability of all the antioxidants in the sample to reduce Cu^{++} to Cu^+ was applied as an index of the sample's antioxidant capacity. The antioxidant concentrations of the samples were then determined by further extrapolation from a standard curve developed from known concentrations of uric acid (36). The procedure supplied with the assay was followed.

5.5.5 Mercury Analysis

Total Mercury (THg) concentrations were determined on hair samples (0.2 grams) collected with stainless-steel surgical scissors from the front dorsal area of the neck near the skin. Hair samples were washed prior to digestion. THg concentration in the hair samples were measured at Frontier Geosciences (Seattle, WA) using the cold vapor atomic fluorescence spectrometry (CVAF) method (10). The results are reported on a wet weight (w/wt) basis as ng/g (ppb).

5.5.6 Statistical Analysis

Samples were analyzed using SAS statistical software. Analysis of variance was performed on all parameters to evaluate the effects of sampling location on the measured index. One sample was removed in the Salcha group for mercury because the sample was more than four times the mean (34). Significant differences between sampling sites was determined using Tukey's Studentized range test. Linear regression models were performed using all combinations of variables to determine any correlation between variables. Differences were considered significant at $P \leq 0.05$.

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5.7 References

1. Belanger MC, Dewailly E, Berthiaume L, Noël M, Bergeron J, Mirault ME, Julien P (2006) *Metabolism* 55; 989-95.
2. Mozaffarian D, Rimm EB (2006) *JAMA* 296(15): 1885-99.
3. Tyrell M (2006) *Arctic* 59(4): 370-80.
4. Strasser A, Niedermuller H, Hofecker G, Laber G (1993) *Zentralbl Veterinarmed A* 40: 720-730.
5. AdamsB, Chan A, Callahan H, Siwak C, Tapp D, Ikeda-Douglas C, Atkinson P, Head E, Cotman C, Milgram N (2000) *Behav Brain Res* 108: 47-56.
6. Greeley EH, Ballam JM, Harrison JM, Kealy RD, Lawler DF, Segre M (2001) *Vet Immunol Immunopathol* 82: 57-71.
7. Milgram NW, Zicker S, Head E, Muggenburg B, Murphey H, Ikeda-Douglas C, Cotman C (2002) *Neurobiology of Aging* 23: 737-745.
8. Balagangatharathilagar M, Swarup D, Patra RC, Dwivedi SK (2006) *Sci Total Environ* 359: 130-4.
9. Hansen J, Danscher G (1995) *Pharmacol Toxicol* 77: 189-195.
10. Dunlap KL, Reynolds AJ, Duffy LK (2007) Submitted *Sci Total Environ.*
11. Andersen DB (2002) Alaska Department of Fish and Game Technical Paper No. 210.
12. Bang HO, Dyerberg J, Nielsen AB (1971) *Lancet* 1: 1143-1145.
13. Dyerberg J, Bang HO, Hjorne N (1975) *Am J Clin Nutr* 28: 958-966.
14. Lanier AP, Kelly JJ, Maxwell J, McEvoy T, Homan C (2006) *Alaska Med* 48(2): 30-3.

15. Martinsen N, Jørgensen ME, Bjerregaard P, Krasnik A, Carstensen B, Borch-Johnsen K (2006) *Int J Circumpolar Health* 65(3): 243-52.

16. Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG, Pascoe WS (1987) *Science* 237: 885-888.

17. Adler AI, Boyko EJ, Schraer CD Murphy NJ (1994) *Diabetes Care* 17: 1498-1501.

18. McGrath-Hanna N, Greene D, Tavernier R, Bult-Ito A (2003) *Int J Circumpolar Health* 62: 228-241.

19. Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Caan B, Packer L (2002) *Am J Epidemiol* 156: 274-285.

20. Dogra G, Ward N, Croft KD, Mori TA, Barrett PH, Herrmann SE, Irish AB, Watts GF (2001) *Nephrol Dial Transplant* 16: 1626-1630.

21. Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC, Miles EA, Ballinger AB, Thompson RL, Calder PC (2003) *Br J Nutr* 90: 405-412.

22. Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA (2002) *Br J Nutr* 87(Suppl 1): S31-S48.

23. Philpott M, Ferguson LR (2004) *Mut Res* 551: 29-42.

24. Simopoulos AP (2002) *J Am Coll Nutr* 21(6): 495-505.

25. Meydani SN, Santos MS (1999) in *Nutrition and Immunology: Principles and Practice*, eds Gershwin ME, German B, Keen C (Humana Press), pp 403-421.

26. Ciubotaru I, Lee Y, Wander RC (2003) *J Nutr Biochem* 14: 513-521.

27. Lehr HA, Hübner C, Nolte D, Kohlshütter A, Messmer K (1991) *Proc Natl Acad Sci* 88: 6726-30.

28. Croft KD, Codde JP, Barden A, Vandongen R, Beilin LJ (1988) *Clin Exp Pharmacol Physiol* 15(7): 517-25.

29. Volanakis JE (2001) *Mol Immunol* 38: 189-197.

30. Hild CM (1998) *Int J Circumpolar Health* 57 Suppl(1): 561-566.

31. Gamberg M, Braune BM (1999) *Sci Tot Environ* 243/244: 329-38.

32. Hoekstra PF, Braune BM, Elkin B, Armstrong FJ, Muir DG (2003) *Sci Tot Environ* 309: 81-92.

33. Krauss C (2004) *NY Times (Print)* A(1): 4.

34. Laflamme D (1997) *Canine Practice* 22: 10-15.

35. Reynolds AJ, Reinhart GA, Carey DP, Simmerman DA, Frank DA, Kallfelz FA (1999) *Am J Vet Res* 60: 789-795.

36. Dunlap KL, Reynolds AJ, Duffy LK (2006) *Comp Biochem Physiol A Mol Integr Physiol* 143(4): 429-34.

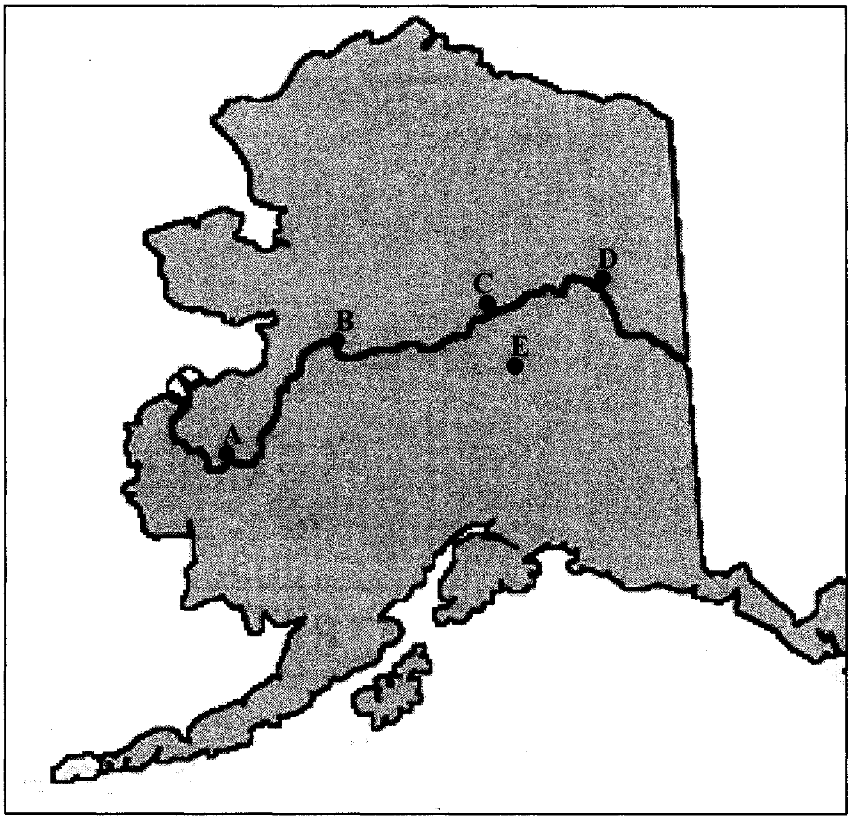

Figure 5.1: Locations of the sample sites along the Yukon River in the state of Alaska. (A) represents Russian Mission, (B) represents Galena, (C) represents Rampart, (D) represents Fort Yukon, and (E) represents Salcha.

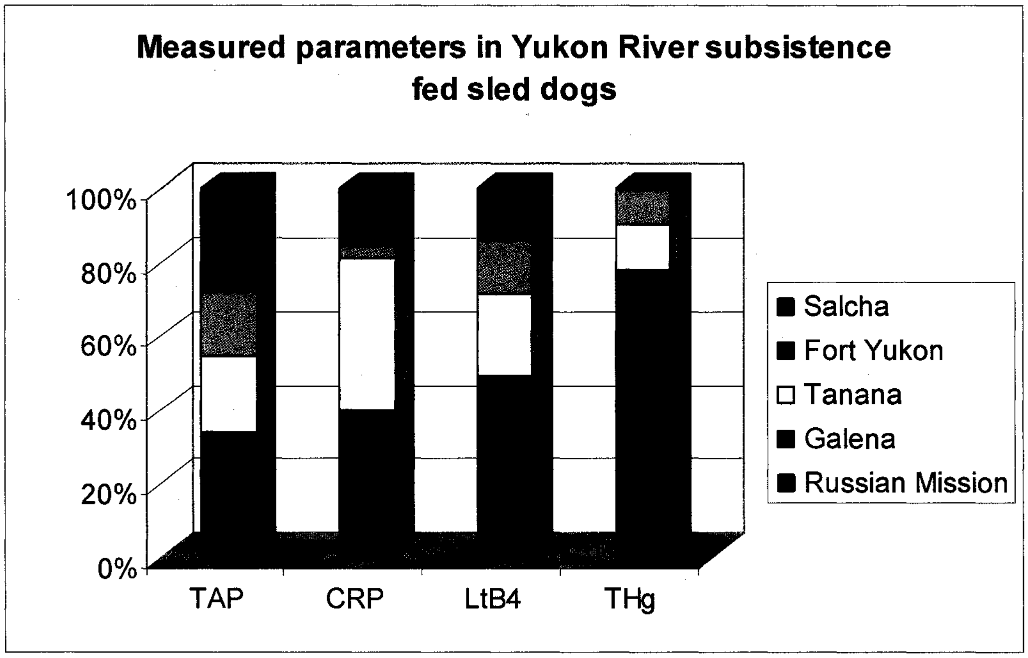

Figure 5.2: Percent of TAP, CRP, LtB_4, and THg that each village contributed to the total for each biomarker measured.

Table 5.1: TAP, CRP, LtB_4, and THg concentrations in village sled dogs

Variable Village	Village Russian Mission	Galena	Rampart	Fort Yukon	Salcha
TAP mean (mM)	**0.133±0.04**	**0.122±0.03**	**0.152±0.03**	**0.131±0.01**	**0.208±0.07**
Russian Mission	0.000				
Galena	0.011	0.000			
Rampart	-0.019	-0.030	0.000		
Fort Yukon	0.002	-0.009	0.021	0.000	
Salcha	-0.075*	-0.086*	-0.056*	-0.077*	0.00
CRP mean (pg/ml)	**14.72±15.07**	**7.67±10.37**	**23.19±9.93**	**2.03±2.26**	**8.64±11.72**
Russian Mission	0.00				
Galena	7.05	0.00			
Rampart	-8.45	-15.52*	0.00		
Fort Yukon	12.69	5.64	21.16*	0.00	
Salcha	6.08	-0.97	14.55*	-6.61	0.00
LtB_4 mean (pg/ml)	**15.75±5.03**	**28.19±11.78**	**19.59±7.45**	**12.93±2.42**	**12.55±3.14**
Russian Mission	0.00				
Galena	-12.44*	0.00			
Rampart	-3.84	8.60*	0.00		
Fort Yukon	2.83	15.26*	6.66	0.00	
Salcha	3.20	15.64*	7.04	0.38	0.00
THg mean (ng/g)	**10908.3±3028**	**4528.2±1125**	**2446.8±1737**	**1822.4±1747**	**91.1±18.6**
Russian Mission	0.0				
Galena	6380.1*	0.0			
Rampart	8461.5*	2081.4	0.0		
Fort Yukon	9085.9*	2705.8*	624.4	0.0	
Salcha	10817.2*	4437.1*	2355.7*	1731.3	0.0

This table shows mean concentrations and standard deviations for each biomarker measured. Differences in mean concentrations between each village are shown and significant differences of $P < 0.05$ are indicated with an asterisks (*).

Chapter 6

Future Directions

6.1 Melatonin

Melatonin synthesis is inhibited by light on the mammalian retina and peaks in plasma concentrations occur during the night. There was not a significant difference in melatonin levels in the winter in Alaskan sled dogs between 2:00 and 8:00, unlike New York and Alaska summer sled dogs. This suggests that in the winter in the Arctic there is a longer duration of melatonin secretion and/or the peak in melatonin production is shifted to the right. A shift in peak would infer an adaptation to the light cycle and longer duration of melatonin secretion would be a consequence of night length. In order to determine which conclusion is made from the data obtained, more sample times are necessary between 2:00 and 8:00. Because of exceptionally long night length in Fairbanks, Alaska, it would be interesting to compare melatonin levels in smaller intervals from 15:00 on one day to 11:00 on the next.

6.2 Thyroid hormones

Metabolic rate and thermoregulation are key factors influenced by thyroid hormones. Exercise has shown to reduce thyroid hormone production in sled dogs (Panciera et al., 2003; Lee et al., 2004). Data presented here supported a temporary suppression of thyroid hormone production as a result of exercise. The events in which Lee et al. (2004) measured thyroid hormone production in sled dogs is one of the most extreme and publicized endurance races in the world: the 1,100 mile Iditarod sled dog race. Sled dogs participating in these experiments were sprint type huskies. Though all sled dogs are genetically similar, one type of sled dog is an endurance athlete, while the other is a speed athlete. Depending on the duration of the event, it is difficult to ascertain which type of exercise is more physically demanding. In terms of thyroid hormone production, this poses some interesting questions; what type of exercise has more of an impact on thyroid hormone production, and how long after an extreme exercise bout of both types, does thyroid function return to normal? This could easily be tested by sampling dogs from the Iditarod and dogs from one of the most extreme sprint races, such as the Open North American, before, immediately after, and multiple post-race samples. This would also have interesting ramifications for human athletes. Exercise has many benefits, but at some level it becomes deleterious. The same holds true for sled dogs. At what intensity or duration does exercise exceed the body's basal regulatory mechanisms?

6.3 Sled dogs as a model for nutritional adaptation in the circumpolar north

We observed varying degrees of contaminant exposure in subsistence fed sled dogs depending on village location. Immune parameters also differed based on location but leukotriene B_4 and C-reactive protein did not correlate with mercury exposure. This may be due to differences in diet or changes in the nutrient composition of salmon as they migrate. In order to create a more complete profile, it is necessary to collect and analyze diet samples for each village. However, antioxidant status negatively correlated with mercury concentration in village sled dogs. Mercury is capable of inducing lipid peroxidation through oxidation of sulfhydryl groups in enzymes, ion channels and receptors, interfering with antioxidants systems, such as glutathione (Belanger et al., 2006; Mozaffarian and Rimm, 2006).

The current project only measured two inflammatory markers, which did not correlate with each other. To get a more extensive understanding of the impacts of mercury exposure on immune function, additional immune parameters should be measured as well as individual lipid profiles. Three important inflammatory cytokines that will be measured in the near future include interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α).

Interleukin-6 is a pleiotropic cytokine produced by both lymphoid and non-lymphoid cells and plays a pivotal role in host defense, acute phase reactions, immune responses, nerve cell function, hematopoiesis, and bone remodeling (Shin et al., 2001; Somers et al., 1997; Varghese et al., 2002; Yamasaki et al., 1988). A number of inflammatory diseases, such as rheumatoid arthritis, glomerular nephritis, and psoriasis, exhibit an over-expression of IL-6 (Shin et al., 2001).

Interleukin-1 and tumor necrosis factor-alpha are also multifunctional cytokines, but the biological activity associated with these two cytokines mimic each other in response to infection and injury. Both are endogenous pyrogens that induce shock and pulmonary hemorrhage and elicit such responses with a synergistic effect. The main difference between the two is that TNF-α has no direct effect on lymphocytes. Individually these cytokines display a wide array of biological effects and are produced and recognized by a variety of cells. The biological roles of IL-1 and TNF-α are quite expansive and include key functions in inflammatory responses, antitumor activity, antiviral processes, and septic shock (Dinarello, 1996; Dinarello, 2000; Gruss and Dower, 1995).

The production of pro-inflammatory cytokines has been shown to be affected by environmental contaminants. Duffy et al (1996) observed significant increases in plasma IL-6 and IL-1 levels of marine mammals affected by the Exxon Oil Valdez spill of 1989. IL-6, IL-1, and TNF-α are soluble, locally released inflammatory mediators produced by monocytes and macrophages following immune activation. T-lymphocytes produce cytokines. Th1 lymphocytes produce pro-inflammatory cytokines, namely IL-2 and IFN-γ that have several immunological effects, one of which is the activation of macrophages. Macrophages in turn produce IL-6, IL-1, and TNF-α (Calder et al., 2002). Although, the production of proinflammatory cytokines is an essential part of response to trauma and infection, excessive production increases the risk of a wide range of diseases (Philpott and Ferguson, 2004).

Lowered prostaglandin (PG) production is associated with the consumption of a diet high in omega-3 fatty acids. PG inhibits lymphocyte proliferation, and the production of Th1 cytokines. In turn, PG inhibits Major histocompatibility complex II expression and therefore the production of TNF-α, IL-1, and IL-6 (Calder et al., 2002; Meydani and Santos, 1999). Omega-3 fatty acids may also have a direct effect on IL-6, IL-1 and TNF-α (Ciubotaru et al., 2003).

Sled dogs have been shown to be a reliable research model for studying biological and physiological effects associated with this unique sub arctic environment. Continued monitoring of reliable biomarkers of immune stress will provide an important stepping stone for understanding the relationship between man and the environment.

6.4 References

Bélanger MC, Dewailly E, Berthiaume L, Noël M, Bergeron J, Mirault ME, Julien P. Dietary contaminants and oxidative stress in Inuit of Nunavik. *Metabolism* 2006; 55: 989-95.

Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. *Br J Nutr* 2002; 87(Suppl 1): S31-S48.

Ciubotaru I, Lee Y, Wander RC. Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT. *J Nutr Biochem* 2003; 14: 513-521.

Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996; 87: 2095-2147.

Dinarello CA. Proinflammatory cytokines. *Chest* 2000; 118: 503-508.

Duffy LK, Bowyer RT, Testa JW, Faro JB. Acute phase proteins and cytokines in alaskan mammals as markers of chronic exposure to environmental pollutants. *American Fisheries Society Symposium* 1996; 18: 809-813.

Gruss HJ, Dower SK. The TNF ligand superfamily and its relevance for human diseases. *Cytokines Mol Ther* 1995; 1(2): 75-105.

Lee JA, Hinchcliff KW, Piercy RJ, Schmidt KE, Nelson S. Effects of racing and nontraining on plasma thyroid hormone concentrations in sled dogs. *J Am Vet Med Assoc* 2004; 224(2): 226-31.

Meydani SN, Santos MS. Aging: Nutrition and immunity, pp. 403-421. In: M.E. Gershwin, B. German, C. Keen, (ed) Nutrition and Immunology: Principles and Practice. 1999 Humana Press, Totowa, NJ.

Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* 2006; 296(15): 1885-99.

Panciera DL, MacEwen EG, Atkins CE, Bosu WT, Refsal KW, Nachreiner RF. Thyroid function tests in euthyroid dogs treated with l-thyroxine. *Am J Vet Res* 1990; 51: 22-26.

Philpott M, Ferguson LR. Immunonutrition and cancer. *Mut Res* 2004; 551: 29-42.

Shin IS, Kim HR, Nam MJ, Youn HY. Studies of cocktail therapy with multiple cytokines for neoplasia or infectious disease of the dog I. cDNA cloning of canine IL-3 and IL-6. *J Vet Sci* 2001; 2: 115-120.

Somers W, Stahl M, Seehra JS. 1.9 A crystal structure of interleukin 6: implications for a novel mode of receptor dimerization and signaling. *Embo J* 1997; 16: 989-997.

Varghese JN, Moritz RL, Lou MZ, Van Donkelaar A, Ji H, Ivancic N, Branson KM, Hall NE, Simpson RJ. Structure of the extracellular domains of the human interleukin-6 receptor alpha -chain. *Proc Natl Acad Sci U S A* 2002; 99: 15959-15964.

Yamasaki K, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T. Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. *Science* 1988; 241: 825-828.